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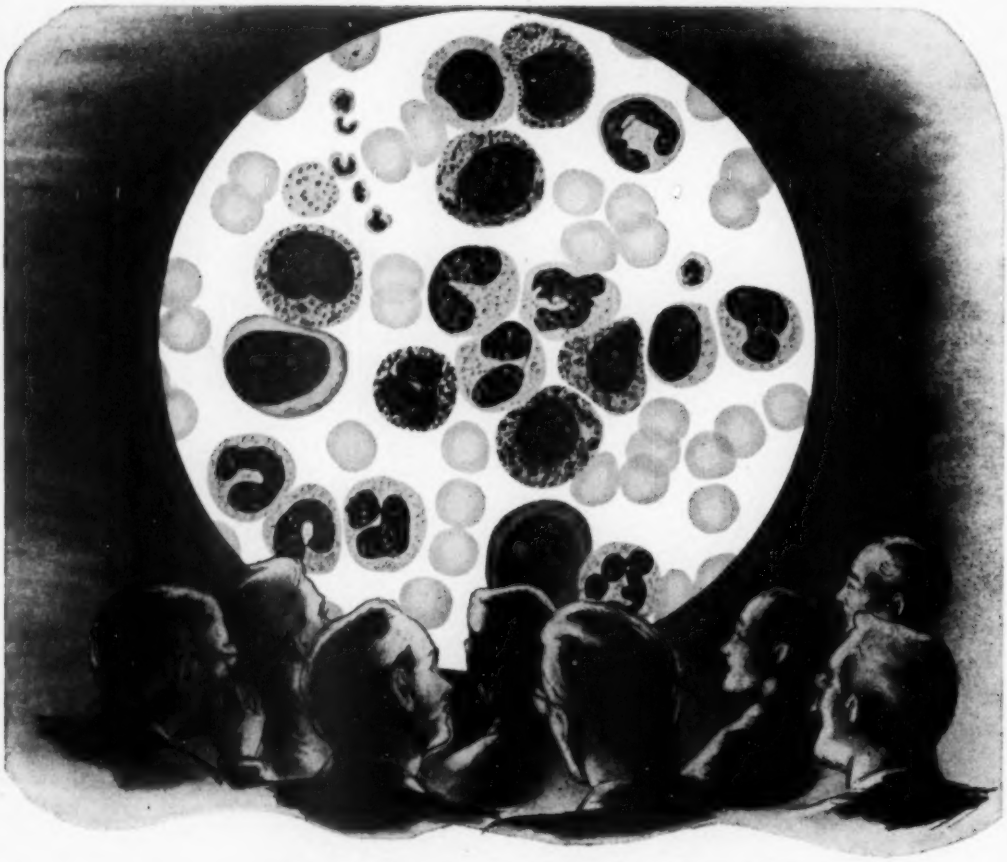
TABLE OF CONTENTS FIRST PAGE



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CONTENTS

Original Articles

Pathogenesis of Coronary Sclerosis PAGE

Paul H. Lober, M.D., Minneapolis 357

Dynamics of Inflammation and of Repair

Virgil H. Moon, M.D., and George A. Tershakovec, M.D., Coral Gables, Fla. 384

Comparison of Pathologic Effects of Radiation in Weanling and Adult Rats

George W. Casarett, Ph.D., Rochester, N. Y. 393

Spontaneous Cryptococcosis of Marmoset Monkeys in Panama

Michael J. Takos, M.D., and Norman W. Elton, M.D., Ancon, Canal Zone 403

Myocarditis in Poliomyelitis

Henry A. Teloh, M.D., Chicago 408

Experimental Aberrant Mediastinal Goiters (Thymic) in the Rat

John H. Van Dyke, Ph.D., Philadelphia 412

Development of Pigmentary Cirrhosis in Cooley's Anemia

John Howell, M.D., and John P. Wyatt, M.D., St. Louis 423

Case Reports

Small Forms of Blastomyces Dermatitidis in Human Tissues

V. Tompkins, M.D., and J. Schleifstein, M.D., Albany, N. Y. 432

Regular Departments

News and Comment 436

Books 436

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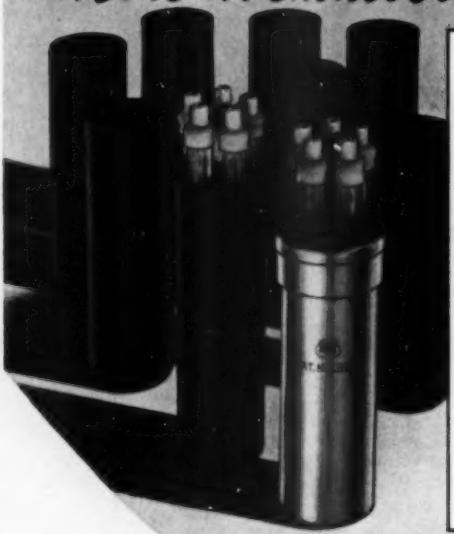

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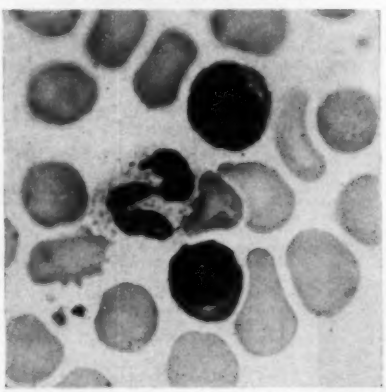

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PATHOGENESIS OF CORONARY SCLEROSIS

PAUL H. LOBER, M.D.
MINNEAPOLIS

ALTHOUGH arteriosclerosis has been studied intensively for over 50 years, it is still poorly understood. This observation is particularly true of coronary atherosclerosis. The investigation of coronary sclerosis in clinical material is hampered by the inaccessibility of the coronary arteries. They cannot be directly examined during life, and only the most indirect means can be used to estimate their condition. Clinical evidence of coronary disease generally occurs only with an advanced process, and then the symptoms may not be proportional to the degree of involvement of the arteries.

The most profitable avenues of investigation remain the use of experimental animals and the study of postmortem material. The results of animal experimentation are not necessarily transferable to humans. The study of postmortem material also offers difficulties. The stage of the disease seen is frequently far advanced, and the actual manner of development of the process must be surmised. The changes in early stages and in otherwise healthy persons cannot be studied routinely. The examination of large numbers of hearts from persons of all ages does give some indication of the course of the disease in the general population.

The study to be reported here is based upon the examination of postmortem material. The primary objective has been determination of the effect of age on the incidence and severity of coronary sclerosis and determination of the age at which the sex difference in incidence may first begin. Other factors which may influence the progress of the disease, such as hypertension, diabetes, and nutrition have also been studied.

Similar studies have been done in the past. Many of these, however, have been incomplete in some aspects of the problem which have been of particular interest.

EFFECTS OF AGE

E. T. Bell [1] has stated that the most prominent influence in the development of arteriosclerosis is age. The commonly accepted belief is that coronary disease develops in later life and that it progresses steadily with age.[2] Coronary sclerosis

From the Department of Pathology, University of Minnesota.

This study was aided by a grant from the National Heart Institute of the United States Public Health Service, of which Dr. Lober was a Fellow.

This paper is an abridgment of a thesis submitted to the Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology.

1. Bell, E. T.: Relation of Arteriosclerosis to Senescence (Discussion), Am. J. Path. **10**: 705, 1934.

2. Piersol, G. M.: Coronary Artery Disease in the Aging, Clinics **4**:1114-1123, 1946.

may affect young persons, however, and Leary [3] thinks this is the best evidence that arteriosclerosis is not a disease of age. White, Edwards, and Dry [4] found no linear progression in severity of coronary sclerosis in men past 50. Clawson and Bell [5] also found no increase in the percentage of those dying of coronary disease beyond the fifth decade.

Holman [6] has suggested that the effects of age are cumulative and the results of multiple episodes. He believes that successive "crops" of lesions can be distinguished in the arteries and that age itself is not the most important factor. Pollak and Wadler [7] also have suggested this episodic process.

Clinically, only 1.7% of all coronary disease occurs before the age of 40.[8] This fact has produced the popular conception that arteriosclerosis is a disease only of more advanced age. Age is evidently an important factor but not always the most important in producing atherosclerosis.

A more difficult aspect of age in relation to coronary disease is the question of the age at which the process starts. Several authors have reported changes occurring in the coronary arteries during the early months of life.[9] The question has been whether these observed changes are pathological or are merely developmental changes in the arterial wall. The changes appear to blend imperceptibly into recognizable atherosclerosis.[10]

SEX DIFFERENCES

One of the most prominent factors influencing coronary sclerosis is sex. Coronary artery disease is remarkably more frequent in males than in females. In series of deaths from coronary disease under 40 years of age, White [11] found 96% and

3. Leary, T.: Arteriosclerosis, Bull. New York Acad. Med. **17**:887-897, 1941.

4. White, N. K.; Edwards, J. E., and Dry, T. J.: The Relationship of the Degree of Coronary Atherosclerosis with Age in Men, Circulation **1**:645-654, 1950.

5. Clawson, B. J., and Bell, E. T.: Incidence of Fatal Coronary Disease in Non-Diabetic and in Diabetic Persons, Arch. Path. **48**:105-106, 1949.

6. Holman, R. L.: Does Arteriosclerosis Develop by Episodic Stages? Am. Heart J. **38**:469, 1949; Studies on the Pathogenesis of Arterial Disease, South. M. J. **42**:108-114, 1949.

7. Pollak, O. J., and Wadler, B.: Studies in Experimental Atherosclerosis: Theoretic and Practical Implications of the Results of Intravascular Injections of Cholesterol Sols, J. Gerontol. **6**:358-364, 1951.

8. Glendy, R. E.; Levine, S. A., and White, P. D.: Coronary Disease in Youth: Comparison of 100 Patients Under 40 with 300 Persons Past 80, J. A. M. A. **109**:1775-1781, 1937.

9. (*a*) Bork, K.: Über Kranzadersklerose, Arch. path. Anat. **262**:646-657, 1926. (*b*) Wolkoff, K.: Über die Atherosklerose der Coronararterien des Herzens, Beitr. path. Anat. **82**:555-596, 1929. (*c*) Ehrich, W.; de la Chapelle, C., and Cohn, A. E.: Anatomical Ontogeny: Man; A Study of the Coronary Arteries, Am. J. Anat. **49**:241-282, 1931. (*d*) Moschcowitz, E.: Hyperplastic Arteriosclerosis Versus Atherosclerosis, J. A. M. A. **143**:861-865, 1950.

10. (*a*) Schmidtmann, M.: Das Vorkommen der Arteriosklerose bei Jugendlichen und seine Bedeutung fur die Ätiologie des Leidens, Arch. path. Anat. **255**:206-272, 1925. (*b*) Hirsch, S.: L'athérome aortique des enfants: Études à propos des débuts de la soi-disant arteriosclerose, Cardiologia **5**:122-188, 1942. (*c*) Fangman, R. J., and Hellwig, C. A.: Histology of Coronary Arteries in Newborn Infants, Am. J. Path. **23**:901, 1947. (*d*) Minkowski, W. L.: The Coronary Arteries of Infants, Am. J. M. Sc. **214**:623-629, 1947. (*e*) Casarini, A.: Osservazioni sulle alterazioni dell' intima coronarica presenti alla nascita, Biol. latina **3**:68-84, 1950.

11. White, P. D.: Coronary Heart Disease in Mid-Century with a Note Concerning Its Military Importance, U. S. Armed Forces M. J. **2**:357-364, 1951.

97% in males. Master, Dack, and Jaffe [12] have also found a high percentage of clinical coronary disease in males. Studies of postmortem series show similar ratios of male preponderance.[13] Females under the age of 50 with coronary disease are unusual.[14]

The reason for the preponderance of males with severe coronary sclerosis and clinical coronary disease is still not clear. Various possibilities have been suggested. Hormonal differences have been considered, and some experimental work has been done.[15] Clinical evidence of hormonal influence is incomplete.[16] Dock [17] has suggested that there is a structural difference between the coronary arteries of males and those of females observable even in the newborn. Others have also shown differences early in life,[10c,d,e] although Zinck [18] considers the observed changes to be physiological. Sociological differences between men and women have been suggested as factors in coronary atherosclerosis.[19]

It is clear that the basis for the sex difference has not been established. A notable fact is that among diabetics the sex difference is practically abolished.[20]

12. Master, A. M.; Dack, S., and Jaffe, H. L.: Age, Sex, and Hypertension in Myocardial Infarction Due to Coronary Occlusion, Arch. Int. Med. **64**:767-786, 1939.

13. (*a*) Clawson, B. J.: Coronary Sclerosis: An Analysis of 928 Cases, Am. Heart J. **17**: 387-400, 1939. (*b*) Gordon, W. H.; Bland, E. F., and White, P. D.: Coronary Artery Disease Analyzed Postmortem, ibid. **17**:10-14, 1939. (*c*) Ackerman, R. F.; Dry, T. J., and Edwards, J. E.: Relationship of Various Factors to the Degree of Coronary Atherosclerosis in Women, Circulation **1**:1345-1354, 1950. (*d*) Willius, F. A.; Smith, H. L., and Sprague, P. H.: A Study of Coronary and Aortic Sclerosis: Incidence and Degree in 5,060 Consecutive Postmortem Examinations, Proc. Staff Meet., Mayo Clin. **8**:140-144, 1933.

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15. Ludden, J. B., Bruger, M., and Wright, I. S.: Experimental Atherosclerosis: Effect of Testosterone Propionate and Estradiol Dipropionate on Experimental Atherosclerosis in Rabbits, Arch. Path. **33**:58-62, 1942. Bruger, M.; Wright, I. S., and Wiland, J.: Experimental Atherosclerosis: Effect of Testosterone Propionate and Estradiol Dipropionate on the Cholesterol Content of the Blood and Aorta in Castrate Female Rabbits, ibid. **36**:612-614, 1943. Horlick, L., and Katz, L. N.: The Effect of Diethylstilbestrol on Blood Lipids and the Development of Atherosclerosis in Chickens on a Normal and Low Fat Diet, J. Lab. & Clin. Med. **33**:733-742, 1948. Pick, R.; Stamler, J.; Rodbard, S., and Katz, L. N.: The Inhibition of Coronary Atheromatosis in Cholesterol-Fed Chicks Receiving Estrogens, Circulation **4**:468, 1951.

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17. Dock, W.: The Predilection of Atherosclerosis for the Coronary Arteries, J. A. M. A. **131**:875-878, 1946.

18. Zinck, K. H.: Sondervorrichtungen an Kranzgefässen und ihre Beziehung zu Coronarinfarkt und miliaren Nekrosen, Arch. path. Anat. **305**:288-297, 1939.

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HYPERTENSION

Elevation of blood pressure has been termed "the most common associated finding" in coronary disease.[21] Clinical coronary disease is closely associated with hypertension.[22] Postmortem studies have shown a similar relationship.[23] Hypertension appears to increase the incidence of coronary disease proportionately in both sexes.[24] Another cause of cardiac hypertrophy—aortic stenosis—is found to be inversely related to the degree of coronary sclerosis.[25]

White, Edwards, and Dry [4] concluded from their study that hypertension was not a primary factor in the production of coronary sclerosis in men. On the other hand, Ackerman, Dry, and Edwards [13c] found that hypertrophied hearts of women had significantly higher degrees of coronary sclerosis than normal-sized hearts. It appears that elevated intravascular pressure tends to accelerate atherosclerosis.

DIABETES MELLITUS

One other factor which influences intimal arterial disease is diabetes mellitus. Clawson and Bell [5] found fatal coronary disease to be twice as frequent in diabetic as in nondiabetic males and three times as frequent in diabetic as in nondiabetic females. Diabetes itself tends to cause clinical coronary disease to develop at an earlier age than in the nondiabetic person. This fact has been related by numerous workers to a disturbance of lipoids in the blood.[26]

EFFECTS OF NUTRITION AND MALIGNANCY

A further factor which may have significance in the development of coronary sclerosis is nutrition. A relationship between overweight and severity of coronary sclerosis has been observed.[27] Ackerman, Dry, and Edwards [13c] found less sclerosis in underweight women.

If nutrition plays a part in the causation of atherosclerosis, it seems possible that changes in the diet may have some effect on the course of the disease. Experimental lesions have been thought to regress on return to normal diets.[28] Another

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21. Gregg, D. E.: Coronary Circulation in Health and Disease, Philadelphia, Lea & Febiger, 1950.

22. Master, Dack, and Jaffe.[12] Levy and Boas.[14a]

23. Bell, E. T., and Clawson, B. J.: Primary (Essential) Hypertension: A Study of 420 Cases, Arch. Path. **5**:939-1002, 1928.

24. Davis, D., and Klainer, M. J.: Studies in Hypertensive Heart Disease: Incidence of Coronary Atherosclerosis in Cases of Essential Hypertension, Am. Heart J. **19**:185-192; Role in the Development of Coronary Sclerosis, ibid., 193-197, 1940.

25. Horan, M. J., and Barnes, A. R.: Calcareous Aortic Stenosis and Coronary Artery Disease, Am. J. M. Sc. **215**:451-455, 1948.

26. Zinn, W. J., and Griffith, G. C.: A Study of Serum Fat Globules in Atherosclerotic and Non-Atherosclerotic Male Subjects, Am. J. M. Sc. **220**:597-603, 1950.

27. (*a*) Dublin, L. I.: The Influence of Weight on Certain Causes of Death, Human Biol. **2**:159-184, 1930. (*b*) Wilens, S. L.: Bearing of General Nutritional State on Atherosclerosis, Arch. Int. Med. **79**:129-147, 1947.

28. Morrison, L. M., and Rossi, A.: Absorption of Aortic Atherosclerosis by Choline Feeding, Proc. Soc. Exper. Biol. & Med. **69**:283-284, 1948. Bevans, M.; Davidson, J. D., and Kendall, F. E.: Regression of Lesions in Canine Arteriosclerosis, A. M. A. Arch. Path. **51**: 288-292, 1951.

approach to this problem has been the study of groups of persons with chronically poor nutrition. A less-severe degree of coronary sclerosis has been found in persons dying of prolonged wasting diseases.[29]

OTHER FACTORS

Other general factors which may play a role in the severity of coronary sclerosis are beyond the scope of the present study. These might include hereditary influences, racial factors, endocrine disturbances, dietary deficiencies, infections, occupation, and habits.

PERSONAL INVESTIGATIONS

The chief purpose of this study was to determine whether coronary sclerosis is a process which is slowly progressive throughout life or whether it advances by steps or stages, developing more rapidly in certain periods of life. It was anticipated that it would be possible to determine the age at which the sex difference in the severity of coronary sclerosis begins as well as the effects of hypertension, diabetes, and possibly nutrition on the process.

The investigation was based on thorough and accurate study of the coronary arteries in a series of hearts removed at autopsy from persons of every age group, so that the progress of the disease in a cross section of the population could be demonstrated. Although this study would not, of course, show the progressive changes in each individual, it was felt that the course of the disease in an individual member of the group might be inferred from the development of the process in the group as a whole.

Previous studies, such as those of Willius, Smith, and Sprague [13d] or Gordon, Bland, and White,[13b] of large series of postmortem examinations to determine the incidence and severity of coronary disease have relied mainly on gross observations and descriptions of the coronary arteries made by a number of pathologists. Grading of the severity of the process under these circumstances must necessarily be somewhat inaccurate, and changing techniques over a long period of years may contribute to the inaccuracy.

Studies such as those of Ackerman, Dry, and Edwards [13c] and White, Edwards, and Dry[4] are exceptions, in that all the arteries have been graded by one person and according to a uniform microscopic standard. These studies were done, however, only on persons over the age of 30. Other series have included only fatal cases of coronary disease [30] or only certain age groups.[10d]

The number of cases used in the present study was selected to provide enough cases in each group for statistical analysis, if possible, yet not too many to prevent careful examination of each heart by the same person.

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29. (*a*) Rosenthal, S. R.: Studies in Atherosclerosis: Chemical, Experimental, and Morphologic; Roles of Cholesterol Metabolism, Blood Pressure and Structure of Aorta (F. A. A.); Infiltration Expression Theory of Lipid Deposit, Arch. Path. **18**:473-506, 1934. (*b*) Wilens, S. L.: The Resorption of Arterial Atheromatous Deposits in Wasting Disease, Am. J. Path. **23**:793-804, 1947. (*c*) Wanscher, O.; Clemmesen, J., and Nielsen, A.: Negative Correlation Between Atherosclerosis and Carcinoma, Brit. J. Cancer **5**:172-174, 1951.

30. Clawson, B. J.: Incidence of Types of Heart Disease Among 30,265 Autopsies, with Special Reference to Age and Sex, Am. Heart J. **22**:607-624, 1941.

MATERIAL AND METHODS

The study consisted of a thorough examination of 536 hearts removed at postmortem examination during 1949, 1950, and 1951. These were collected from autopsies performed by the Fellows of the Pathology Department of the University of Minnesota. A considerable number were obtained from the Ancker Hospital in St. Paul. These hearts were taken at random, without regard for cause of death, except that particular attempts were made to obtain hearts from persons killed by accidental means and from persons in the younger age groups, in order to obtain adequate numbers in these groups for significant comparison. The autopsy protocols of these persons were examined to determine age, sex, body weight, height, cause of death, evidence of hypertension or diabetes, and symptoms or signs of heart disease.

The hearts were opened by the pathologist performing the postmortem examination and were thereafter fixed and preserved in neutral 10% formalin. Each of the three major branches of the coronary arteries, the right coronary, the left main and anterior descending branch, and the left circumflex branch, was sectioned transversely at approximately 2-mm. intervals from the orifice at the aorta to the smallest vessel which could be followed grossly. The cuts were made with a sharp blade at right angles to the lumen, and the exposed arterial wall was carefully examined. The sections of the artery thus cut which showed the greatest degree of sclerosis were taken for histological examination. The total length of the artery was measured, and the position of each section taken was recorded by measurement from the orifice or, in the case of the left circumflex branch, from its origin in the main left coronary artery. The positions of branches were also recorded.

At least two histological sections of each of the three main arterial branches were used for routine hematoxylin and eosin preparations. Other blocks were taken for fat or other special stains.

The grading of the severity of the arteriosclerotic process was done on the basis of examination of the histological preparations. The grading was based on one section of each of the three major branches. This particular section was chosen because it showed the most severe degree of sclerosis. It was representative of the most marked area of sclerosis produced in that artery and should not be considered as representing the average degree of sclerosis of the entire branch.

In most pervious series, grading has been based on the gross appearance of the arteries.[13b,d] Ackerman, Dry, and Edwards [13c] and White [4] graded the arteries microscopically on the basis of reduction of the size of the lumen of the artery as compared with the thickness of the wall. Others have used the thickness of the intima as the basis for evaluation. These are easily measured, and determinations can be made with accuracy. The thickening of the intima is not always regarded as a part of the atherosclerotic process, particularly in younger age groups.[31] There may also be well-developed atherosclerotic changes in the arterial wall without any remarkable change in the size of the lumen.

31. (*a*) Wilens, S. L.: The Nature of Diffuse Intimal Thickening of Arteries, Am. J. Path. **27**:825-839, 1951. (*b*) Duff, G. L., and McMillan, G. C.: Pathology of Atherosclerosis, Am. J. Med. **11**:92-108, 1951.

It was felt that it would be desirable, in addition to measurements of the size of the lumen and thickness of the intima, to select criteria for grading which would be as characteristic as possible of atherosclerosis alone. It was also desired to find a method which would indicate the earliest recognizable changes and show the

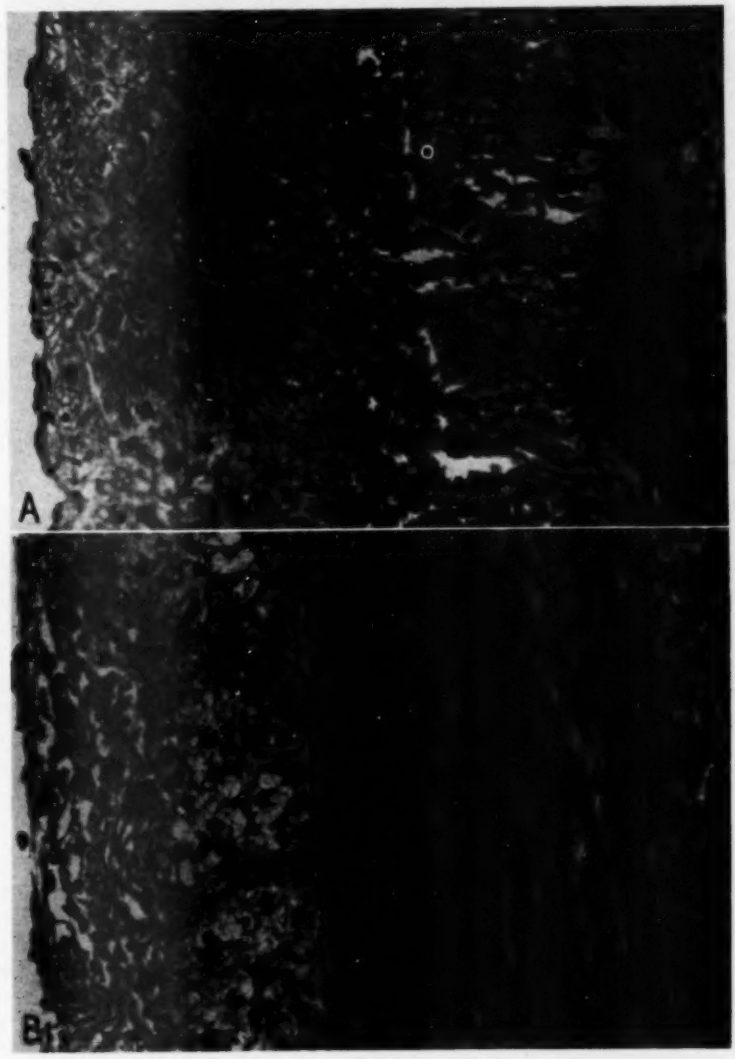

Fig. 1.—*A*, Grade 1 infiltration of intima under high magnification. Note separation of fibers near lumen. (Hematoxylin and eosin, × 120.) *B*, Grade 2 infiltration of intima under high magnification. Loosened appearance extends down to internal elastic lamina. (Hematoxylin and eosin, × 120.)

development of the lesion without regard for its size. The degree of infiltration of the intima was chosen as the criterion best fulfilling these requirements.

Grade 1, on this basis, was defined as the earliest recognizable change in the intima. This was the appearance of a loosening of the structure of the tissue at the luminal surface, with occasional small pockets of homogeneous material between

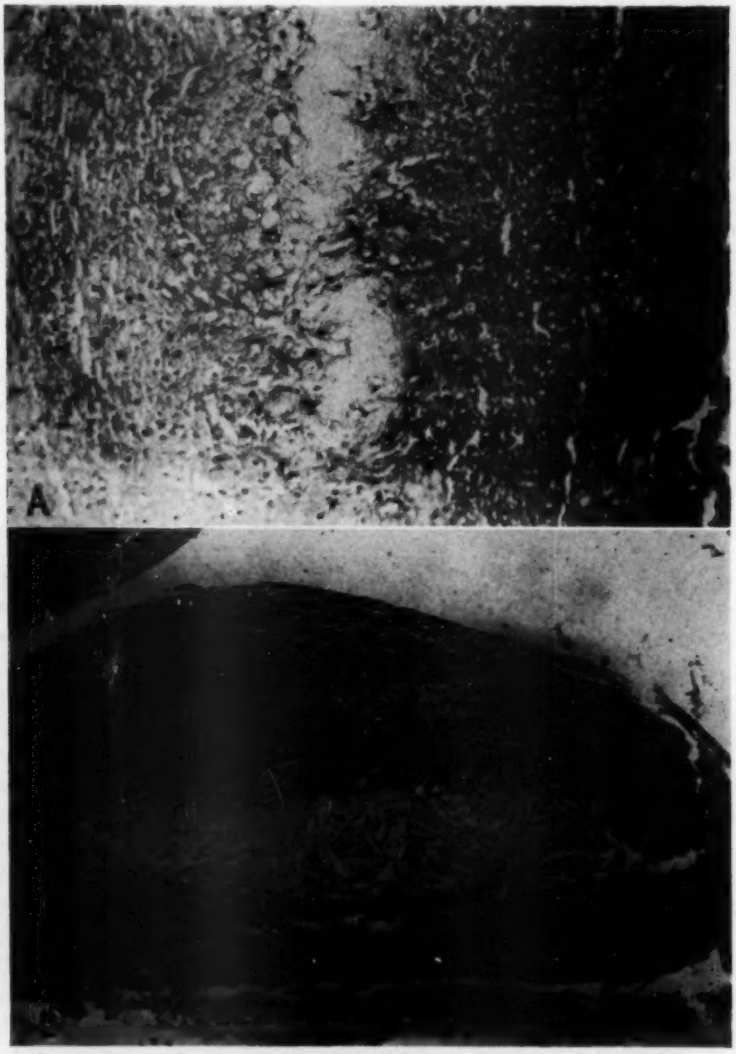

Fig. 2.—*A*, Grade 3 infiltration of intima. There is a broad band of infiltrate deep in the intima. (Hematoxylin and eosin, × 120.) *B*, Grade 4 infiltration of intima. Note large confluent area of infiltrate containing cholesterin crystal clefts. (Hematoxylin and eosin, × 40.)

fibers and very occasional foam cells. The infiltration might extend up to one-half of the distance through the intima. It was difficult to be certain in some cases whether the earliest changes seen might not be artifacts. The appearance with hema-

toxylin and eosin stain was similar to that of edema of the tissue.[32] This change was graded as independently as possible of the thickness of the intima (Fig. 1*A*).

Grade 2 was applied to those arteries which showed the infiltration extending through the deeper layers of the intima up to the internal elastic lamina, with small areas of hyalinization and perhaps a few foam cells near the lumen. Although the intima in these vessels was generally thicker than those of Grade 1, it was not necessarily so (Fig. 1*B*).

Grade 3 was applied to arteries which showed broad streaks of smooth hyaline material in the depths of the intima, often with many foam cells. In addition, there might be separation of fibers in the media with early infiltration there (Fig. 2*A*).

Grade 4 was applied to those sections of artery wall which showed a larger confluent area of hyalinized or necrotic material in the depths of the intima containing cholesterin and often calcium crystals. There might also be large numbers of lipoid-bearing macrophages and often disruption of the media (Fig. 2*B*).

While this scale of grading necessarily required a personal evaluation in every case, all the sections were graded by the same person, so that interpretation of the scale was uniform. There was naturally a wide variation in the appearance of arterial sections, even of those given the same grade, but in every case the basis of the grade was the degree of infiltration, with only supportive evidence from other features, such as foam cells or crystals.

Whether the infiltrate seen was blood plasma,[33] lipoid,[34] or altered protein[35] was not decided. With hematoxylin and eosin staining, this material in the early stages had the appearance of edema fluid. With fat stains, droplets of lipoid were seen early in the process, usually next to the internal elastic lamina, however. Metachromatic dyes stained some of the material.[36] It was impossible to estimate the degree of lipoid infiltration independently of the criteria just described.

It is evident that most investigators would consider the first two grades based on these criteria as early atherosclerosis and would base further grading on the size of the more fully developed atherosclerotic plaque.

The numerical grades of 0 through 4 for each of the three major branches were added and not averaged for each case, so that a numerical grade of 0 through 12 was possible without use of fractions.

The degree of degeneration of the internal lamina was likewise graded independently from 0 through 4. Grade 1 consisted of the first visible splittings of the internal elastic lamina. Grade 2 was applied to those arteries which showed actual breaks in the continuity of the lamina but in which the lamina could be followed around more than one-half of the circumference of the lumen. Grade 3 was applied when less than one-half of the lumen was still surrounded and Grade 4 when no

32. Meyer, W. W.: Die Bedeutung der Eiweissablagerungen in der Histogenese arteriosklerotischer Intimaveränderungen der Aorta, Arch. path. Anat. **316**:268-316, 1949.

33. Sinapius, D.: Zur Genese atherosklerotischer Frühveränderungen der Aorta, Arch. path. Anat. **318**:316-351, 1950. Meyer.[32]

34. Wolkoff.[9b] Wilens.[31a]

35. Gofman, J. W., and others: Lipoproteins and Atherosclerosis, J. Gerontol. **6**:105-119, 1951.

36. Wislocki, G. B.; Bunting, H., and Dempsey, E. W.: Metachromasia in Mammalian Tissues and Its Relationship to Mucopolysaccharides, Am. J. Anat. **81**:1-37, 1947.

recognizable lamina was present. This grading was also done on the sections stained with hematoxylin and eosin, although sections stained for elastic tissue were prepared in many cases in confirmation. These numerical grades for each artery branch were also added, giving a total grade of 0 through 12 for each heart.

The area of the arterial lumen was thought to be a better measure of the decreased functional capacity of the vessel than the diameter. The relative area of the lumen was calculated, the size being expressed as a percentage of the area of the entire artery.

The thickness of the intimal layer was estimated as a fraction of the thickness of the entire arterial wall and this figure was also converted into a percentage. The average outside diameter of each arterial section was measured by micrometer. The last three measurements in each case were averaged for the three arterial branches and not added.

TABLE 1.—*Total Hearts Studied*

Ages	Males	Females	Combined
NB-1 mo.	15	10	25
1-12 mo.	12	12	24
1-9 yr.	29	16	45
10-19	23	18	41
20-29	28	20	48
30-39	31	26	57
40-49	44	30	74
50-59	47	28	75
60-69	42	28	70
70-79	29	25	54
80-89	14	9	23
Total	314	222	536

Statistical evaluation of the results was made by determinations of the standard error of the differences between mean values of the various measurements. Only the standard errors for critical differences are included in the tables.

RESULTS

Distribution of Cases.—A total of 536 hearts were examined, of which 314 were from males and 122 from females. These were divided into groups according to age at death by decades (Table 1). Separate groups were made for newborn infants and for infants of from 1 to 12 months of age. The largest number of cases, 75, was in the 50 to 59 year age group, while the smallest number, 23, was in the 80 to 89 year group. There were insufficient cases in the group past 90 to warrant their inclusion in this study. All the newborn group were mature infants who lived from a few hours to one month.

The proportion of females in this series, 41%, was slightly higher than in the entire University of Minnesota autopsy series, 36%.[5] There were also relatively more young persons in this series than in the entire material. This fact was the result of the special attempt which was made to secure hearts from females and younger persons.

Relation to Age.—When the degree of sclerosis as measured by the degree of infiltration of the intima was compared in the separate age groups, there was seen to be a progressive increase beginning at birth and extending through the seventh decade in both males and females (Table 2). When these figures were plotted in graphic form (Fig. 3*A*), there was seen to be a gradual increase in slope, particularly in the male line, beginning in the fifth decade. The leveling off at the eighth

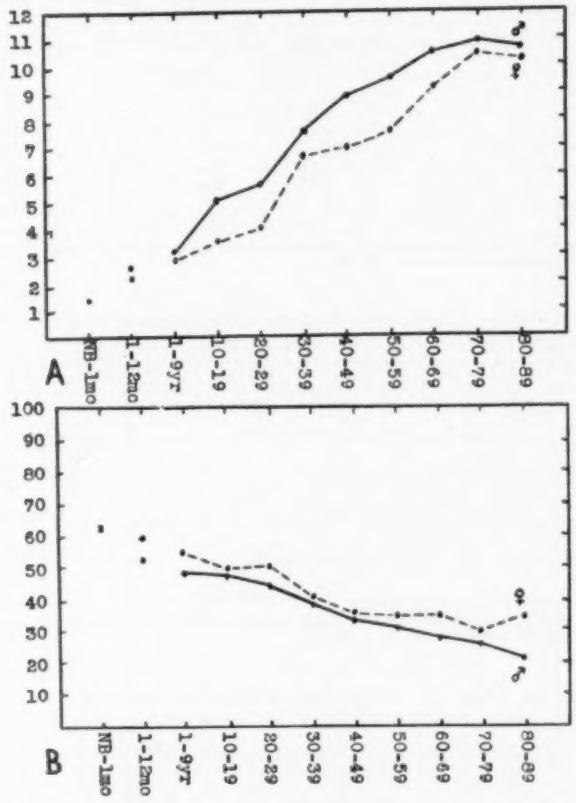

Fig. 3.—*A*, graph showing degree of coronary atherosclerosis as measured by degree of infiltration of intima for each age group. Broken line, representing females, is consistently below that for males. Graded 0 to 12. *B*, graph showing average relative area of lumen of coronary arteries for each age group expressed as percentage of cross-section area of whole artery. Broken line, representing females, shows a consistently larger area than line representing males. Difference between the sexes remains about the same for each age group.

decade appeared to be due to the survival of a few persons with "remarkable hearts." [37] A decline in the older age groups, beginning in the seventh decade in men, was also noted by White, Edwards, and Dry.[4]

37. Willius, F. A., and Smith, H. L.: Further Observations on the Heart in Old Age: A Postmortem Study of 381 Patients Aged 70 Years or More, Am. Heart J. **8**:170-181, 1932.

A similar relationship was seen when the relative area of the lumen was compared (Table 3). There was a progressive decrease in area of the lumen from a high of 62% of the total area of the artery in the newborn to 21% in the ninth-

TABLE 2.—*Average Degree of Atherosclerosis According to Infiltration of Intima (Graded 0 to 12)**

Ages	Males	Females	Combined
NB-1 mo.	1.4	1.4	1.4
1-12 mo.	2.6	2.2	2.4
1-9 yr.	3.1	2.8	3.0
10-19	5.0	3.5	4.3
20-29	5.6	4.0	4.9
30-39	7.6	6.6	7.1
40-49	8.8	6.9	8.0
50-59	9.5	7.5	8.8
60-69	10.4	9.1	9.9
70-79	10.8	10.1	10.6
80-89	10.6	10.2	10.4

* The standard error of the difference between male and female groups is ± 0.57 at age 10 to 19 years. The difference is 2.6 times the standard error at this age.

TABLE 3.—*Average Relative Area of Lumen as Percentage of Area of Artery**

Age	Males, %	Females, %
NB-1 mo.	62	61
1-12 mo.	52	58
1-9 yr.	48	54
10-19	47	49
20-29	44	50
30-39	38	40
40-49	33	35
50-59	30	34
60-69	27	34
70-79	25	29
80-89	21	34

* The standard error of the difference between males and females is ± 2.6 at age 1 to 9 years. The difference is 2.3 times the standard error.

TABLE 4.—*Average Thickness of Intima as Percentage of Thickness of Artery Wall**

Age	Males, %	Females, %
NB-1 mo.	15	12
1-12 mo.	27	15
1-9 yr.	32	26
10-19	43	39
20-29	47	42
30-39	61	57
40-49	68	60
50-59	74	65
60-69	77	71
70-79	80	77
80-89	81	70

* The standard error of the difference between males and females is ± 4.1 at age 1 to 12 months. The difference is 2.9 times the standard error at this age.

decade men (Fig. 3*B*). The relationship was repeated when the figures expressing the thickness of the intima as related to the entire arterial wall were compared (Table 4). The intimal thickness ranged from 12% in newborn girls to 81% in

the ninth-decade men. The curve obtained when these figures were graphed showed a gradually decreasing rate of progression, with the sharpest rise between the third and fourth decades in both sexes (Fig. 4*A*).

When the outside diameter of the artery was plotted in a similar manner, the size ranged from 0.7 mm. in newborn girls to 3.6 mm. in men of the eighth and ninth decades (Table 5, Fig. 4*B*). It was somewhat unexpected to find the size

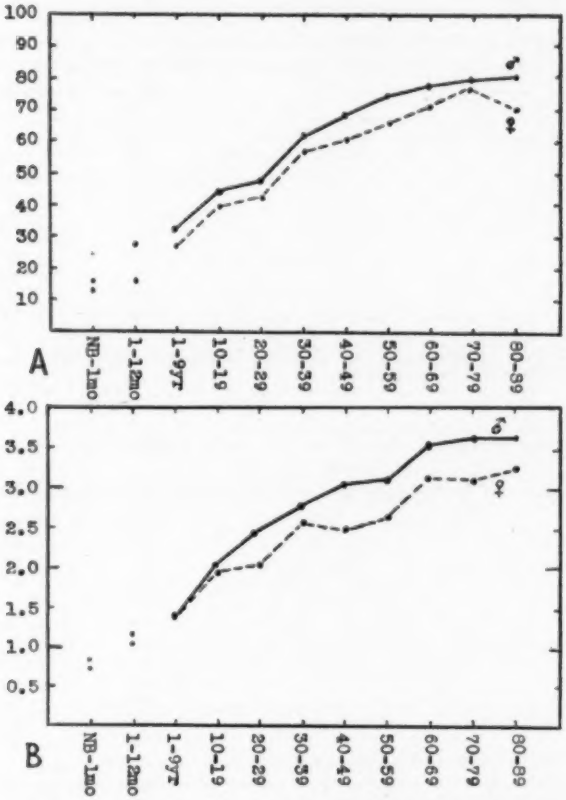

Fig. 4.—*A*, graph showing relative thickness of intima expressed as percentage of thickness of arterial wall averaged for each age group. Broken line represents females and is lower for every group than line for males. *B*, graph showing average outside diameter of coronary arteries for each age group, measured in millimeters. There is a leveling-off for higher age groups. Broken line represents females. There is a progressive increase in sex difference.

of the artery increasing throughout life. A similar increase was noted by Ehrich, de la Chapelle, and Cohn [9c] in the coronary arteries and other vessels. The enlargement possibly represents dilatation or loss of elasticity rather than continued growth, at least in the older age groups.

A comparison of the average degree of elastic-lamina degeneration for each heart showed figures ranging from 3.1 in newborn girls to 9.8 (out of a possible 12) in men in the ninth decade (Table 6). The curve plotted from these figures showed nearly a straight-line relationship (Fig. 5*A*).

Sex Difference.—Almost from the outset, by any of the criteria used for measurement, the males were seen to have considerably more sclerosis in every decade. In the comparison of degrees of sclerosis by infiltration of the intima (Fig. 3*A*), the line representing the females was roughly parallel to the male line

TABLE 5.—*Average Outside Diameter of Coronary Arteries in Millimeters* *

Age	Males, Mm.	Females, Mm.
NB-1 mo	0.8	0.7
1-12 mo	1.1	1.0
1-9 yr	1.3	1.3
10-19	2.0	1.9
20-29	2.4	2.0
30-39	2.7	2.5
40-49	3.0	2.4
50-59	3.1	2.6
60-69	3.5	3.1
70-79	3.6	3.1
80-89	3.6	3.2

* The standard error of the difference between males and females is ± 0.059 at age 20 to 29 years. The difference is 6.7 times the standard error at this age.

TABLE 6.—*Average Degree of Internal Elastic Lamina Degeneration (Graded 0 to 12)* *

Age	Males	Females
NB-1 mo	3.4	3.1
1-12 mo	4.6	3.8
1-9 yr	5.7	4.7
10-19	6.6	6.3
20-29	7.4	6.6
30-39	7.7	7.3
40-49	8.1	6.9
50-59	8.9	6.5
60-69	8.8	7.4
70-79	8.6	8.2
80-89	9.8	8.9

* The standard error of the difference between males and females at ages 40 to 49 years is ± 0.078. The difference is 15 times the standard error at this age.

and approached it most closely in the first and in the eighth and ninth decades. The difference between males and females was not significant, in the sense of being more than twice the standard error of the difference between the means, below the age group 10 through 19 years.

In the data comparing the relative areas of the lumen in males and females (Fig. 3B), there was a statistical significance in the difference in as young a group as 1 through 9 years. When intimal thickness was compared (Fig. 4*A*), there was a mathematical significance in the difference between males and females at the age of 1 through 12 months.

In the comparison of the measurements of the outside diameters of the vessels (Fig. 4*B*), the differences between males and females appeared to increase gradually with age. They were not significant below the age group of 20 through 29 years. In comparison of the degrees of elastic lamina degeneration (Fig. 5*A*), the results were more scattered, and in many decades there was no significant difference between males and females. This appeared to be the least-differentiating measurement.

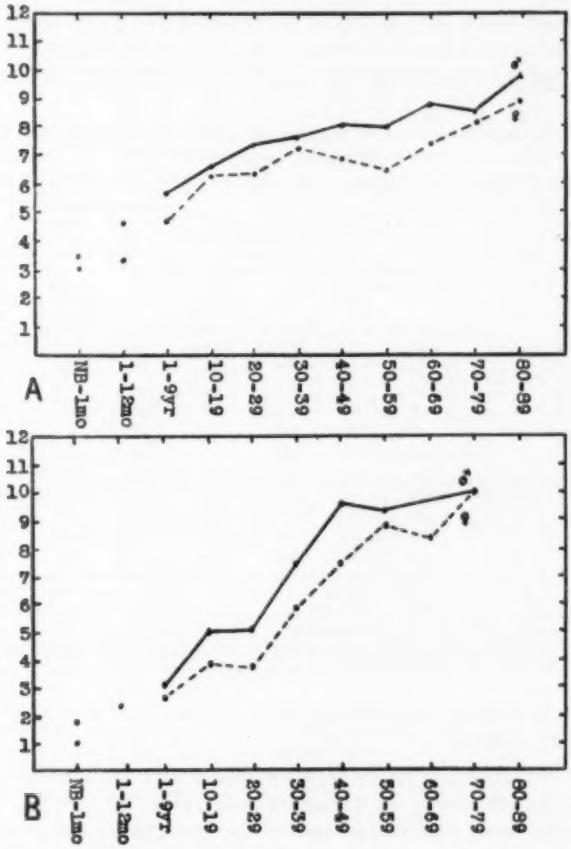

Fig. 5.—*A*, graph showing degree of degeneration of internal elastic lamina, graded 0 to 12. Broken line represents females. *B*, graph showing average degree of coronary sclerosis as measured by infiltration of intima (graded 0 to 12) for a group of deaths from trauma and acute illness. Broken line represents females.

It was evident from this comparison that it was relatively immaterial whether one measured intimal thickness, relative size of lumen, or degree of infiltration of the intima in estimating the degree of coronary sclerosis.

Trauma and Acute Illnesses.—As a control group, to reduce the possibility that there had been inadvertent selection of persons having severe coronary disease,

those who died of traumatic or accidental means were separated from the entire group (Table 7). There were 53 males and 29 females in this group. They ranged in age through nearly the entire age range and consisted of persons killed in traffic accidents, shootings, and other deaths by external means. It was felt that the changes in the coronary arteries should be no greater or less in this group than in the population as a whole and that any possible effects of terminal illness would be eliminated.

In order to obtain more cases in the control group, those persons who died of acute brief illnesses which were unrelated to cardiovascular disease were also added. The degree of coronary sclerosis in this group closely resembled that of persons of corresponding age and sex in the traumatic group (Table 7). The acute-illness

TABLE 7.—*Average Degree of Coronary Sclerosis in Deaths from Trauma and Acute Illness (Graded 0 to 12)**

	Total Cases		Grades of Sclerosis					
			Males, Degree		Females, Degree		Males and Females, Degree	
	Trauma Only	Acute Illness Only	Trauma Only	Combined Trauma & Acute Illness	Trauma Only	Combined Trauma & Acute Illness	Trauma and Acute Illness	Average for Whole Series
NB-1 mo.	8	2	2.2	1.7	1.0	1.0	1.5	1.4
1-12 mo.	0	6	...	2.3	...	2.3	2.3	2.4
1-9 yr.	8	14	3.3	3.1	2.8	2.6	2.9	3.0
10-19	6	5	3.5	5.0	4.0	3.8	4.6	4.3
20-29	14	11	5.4	5.1	4.0	3.7	5.4	4.9
30-39	11	12	8.4	7.5	6.0	5.8	6.6	7.1
40-49	11	5	9.5	9.6	8.0	7.4	8.9	8.0
50-59	13	0	9.3	9.3	8.8	8.8	9.2	8.8
60-69	8	1	9.7	9.7	6.5	8.3	9.6	9.9
70-79	3	0	10.0	10.0	10.0	10.0	10.0	10.6
80-89	0	0	...	...	...	...	...	10.4
Totals	82	56	....	....	....	....	....	....

* The standard error of the difference between the last two columns is ± 0.38 at ages 60 to 69 years. The difference is 0.30, which is within the standard error.

group included 30 males and 26 females, giving a total of 83 males and 55 females in the entire control group.

The degree of coronary sclerosis in this group, as measured by infiltration, gave a curve closely similar to that for the entire series (Fig. 5*B*). When the data for males and females were combined in each of the two groups, the resulting curves nearly overlapped, and there was no significant difference between the two groups (Fig. 6*A*).

Malignant Neoplasms.—Another group, those dying of a malignant neoplasm, was also separated from the main group (Table 8). There were 69 males and 52 females in this group. When the degree of coronary sclerosis in this group was compared with that of the whole series, it was found to be considerably lower (Fig. 6*B*). There was a mathematically significant difference between the two groups. A comparison of the thickness of the intima in these two groups showed a similar difference (Table 9).

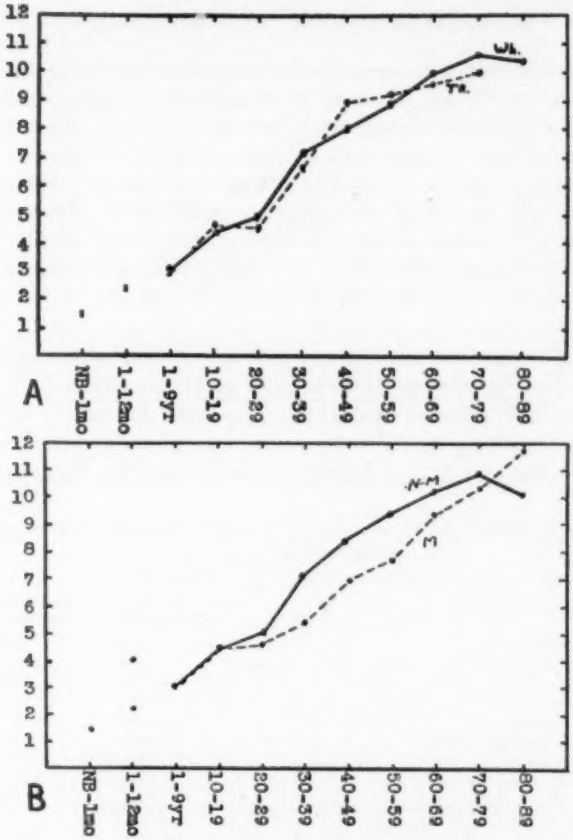

Fig. 6.—*A*, graph showing degree of coronary sclerosis as measured by infiltration of intima (graded 0 to 12) for traumatic and acute-illness group, represented by broken line, compared with that for entire series. The lines overlap, with no significant difference between the values. *B*, graph showing degree of coronary sclerosis as measured by infiltration of intima (graded 0 to 12) for group of deaths associated with malignancy as compared with that for all other deaths. Broken line represents deaths associated with malignancy.

TABLE 8.—*Average Degree of Sclerosis in Death of Malignant Neoplasms (Graded 0 to 12)**

Ages	No. of Cases	Degree	Cases Without Malignancy, Degree
NB-1 mo	0	...	1.4
1-12 mo	2	4.0	2.2
1-9 yr	5	3.0	3.0
10-19	8	4.4	4.4
20-29	5	4.6	5.0
30-39	3	5.3	7.2
40-49	22	6.9	8.5
50-59	25	7.6	9.4
60-69	24	9.3	10.2
70-79	21	10.3	10.8
80-89	6	11.7	10.1
	121		

* The standard error of the difference between cases with and without malignancy is ± 0.66 at ages 40 to 49. The difference is 1.6 or 2.4 times the standard error at this age.

Hypertension.—Those cases were selected from the entire series which showed a heart weight at least 50 gm. in excess of the maximum weight which would be predicted from the individual's height, the figures of Zeek being used.[38] This grouping seemed to be a more accurate basis for selection from the data available than that based on body weight.[39] Hearts which showed evidence of other cardiac disease, such as valvular lesions, which might have produced the hypertrophy were not used. The simpler method of selecting hypertrophied hearts on the basis of an arbitrary weight [40] was not applicable to this group, which included all ages. The normal heart weights of children were obtained from the data of Coppoletta and Wolbach.[41]

There were 77 males and 39 females in the group with hypertrophied hearts. It has been assumed that the hypertrophy in the great majority of these cases was due to hypertension,[12a] although this causation has been disputed by some investigators.[42] All the remaining hearts in the series were placed in a nonhypertensive group, although a few were hypertrophied for reasons other than hypertension.

TABLE 9.—*Average Thickness of Intima in Death of Malignant Neoplasms (in Percentage of Artery Wall)*

Ages	Deaths with Malignancy	Deaths Without Malignancy
NB-1 mo	..	14%
1-12 mo	40%	18
1-9 yr	28	30
10-19	37	42
20-29	39	45
30-39	50	60
40-49	61	66
50-59	68	72
60-69	74	74
70-79	77	80
80-89	78	76

A comparison of the degree of coronary sclerosis in the two groups (Table 10) showed that there was a remarkable difference between them, especially in the age range of 30 to 49 years (Fig. *7A*). Removal of the hypertensive hearts from the entire group did not appear to influence appreciably the progressive increase in the degree of sclerosis with age in the nonhypertensive group, and it had no effect on the sex ratio of the remaining cases. The curve for hypertensives appeared to be flattened, owing to the fact that the degree of sclerosis was nearly at the maximum measurable, even in the lower age groups.

38. Zeek, P.: Heart Weight: Weight of the Normal Human Heart, Arch. Path. **34**:820-832, 1942.

39. Smith, H. L.: The Relation of Weight of the Heart to the Weight of the Body, and the Weight of the Heart to Age, Am. Heart J. **4**:79-93, 1928.

40. Ackerman, Dry, and Edwards.[12c] Clawson.[30]

41. Coppoletta, J. M., and Wolbach, S. B.: Body Length and Normal Weights of Organs Between Birth and 12 Years of Age, Am. J. Path. **9**:55, 1935.

42. Davis, D., and Blumgart, H. L.: Cardiac Hypertrophy: Its Relation to Coronary Arteriosclerosis and Congestive Heart Failure, Ann. Int. Med. **11**:1024-1038, 1937.

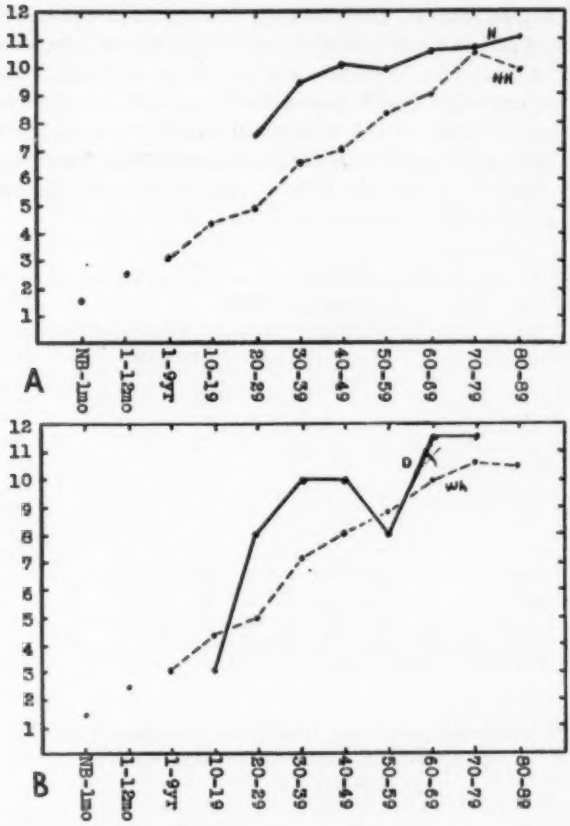

Fig. 7.—*A*, graph showing average degree of coronary sclerosis as measured by infiltration of intima (graded 0 to 12) for hearts with hypertrophy due to hypertension as compared with all other hearts. Unbroken line represents hypertensive group. *B*, graph showing average degree of coronary sclerosis as measured by infiltration of intima (graded 0 to 12) in 18 cases of diabetes as compared with that of entire series. Unbroken line represents diabetics.

TABLE 10.—*Average Degree of Sclerosis in Hearts with Hypertrophy Due to Hypertension (Graded 0 to 12)**

	Males		Females		Males and Females	
	Cases	Degree	Cases	Degree	Cases	Degree
20-29	2	7.5	0	...	2	7.5
30-39	8	8.9	5	10.2	13	9.4
40-49	18	10.1	4	10.3	22	10.1
50-59	16	10.3	4	8.1	20	9.9
60-69	21	11.0	12	10.0	33	10.6
70-79	9	11.0	10	10.4	19	10.7
80-89	3	11.7	4	10.7	7	11.1
Total	77		39		116	

* The standard error of the difference between hypertensives and the whole group at age 40 to 49 is ± 0.63. The difference is 2.1 or 3.3 times the standard error.

Diabetes.—Only 18 cases of diabetes were included in this group (Table 11). When the degree of coronary sclerosis of this group was plotted and compared with that of the entire group (Fig. 7*B*), there was seen to be an irregular increase with diabetes which had statistical significance in the fifth decade. Seven of the diabetics also had hypertrophied hearts. With removal of these 7 from the diabetic group, leaving only 11, the curve remained approximately the same. There were too few diabetics in this series to permit any reliable conclusions about the effect of this disease on the atherosclerotic process.

TABLE 11.—*Average Degree of Sclerosis in Diabetics (Graded 0 to 12)**

	Diabetics		
	Total Cases	Degree	Whole Group
10-19 yr.	1	3	4.3
20-29	1	8	4.9
30-39	3	10	7.1
40-49	4	10	8.0
50-59	1	8	8.8
60-69	4	11.5	9.9
70-79	4	11.5	10.6
80-89	0	..	10.4
Total	18		

* The standard error of the difference between the diabetics and the whole group is ± 1.0 at age 40 to 49 years. The difference is 2.0 or just twice the standard error.

TABLE 12.—*Average Degree of Sclerosis and Relative Area of Lumen (Percentage) in Cases of Death from or Symptoms of Coronary Disease (Graded 0 to 12)**

		Males and Females	
Age	Cases	Degree	Lumen
20-29 yr.	1	10	26%
30-39	2	10.5	23.5
40-49	6	11.2	15.8
50-59	10	10.9	20.7
60-69	11	11.6	19.8
70-79	5	11.6	15.6
80-89	1	12	6.0

* The standard error of the difference in degree of sclerosis at age 40 to 49 between the whole group and the coronary-disease group is ± 0.48. The difference is 3.2 or 6.7 times the standard error.

Clinical Coronary Disease.—In order to determine the severity of sclerosis required for clinical signs, the cases of persons having symptoms of, or death from, coronary sclerosis were ranged against the entire group (Table 12). Most of these persons died of the disease. There were 30 men and 6 women, or 6.7% of the entire series. The sex ratio was 3.5 to 1 when corrected for numbers. These figures are similar to those of Clawson.[13a] Three of the persons were below 40 years of age. It was seen from the plotted graph (Fig. 8*A*) that this group had very severe sclerosis of the coronary arteries, usually of all three branches. This observation

suggested that severe disease of all branches was necessary before symptoms were produced. A similar relationship was seen when the area of the lumen was used for comparison (Fig. 8*B*).

It was evident that the curve for males of the entire series came closer to the danger area of clinical coronary disease than that for females. This comparison is one way of showing the increased susceptibility of males to clinical symptoms of coronary disease.

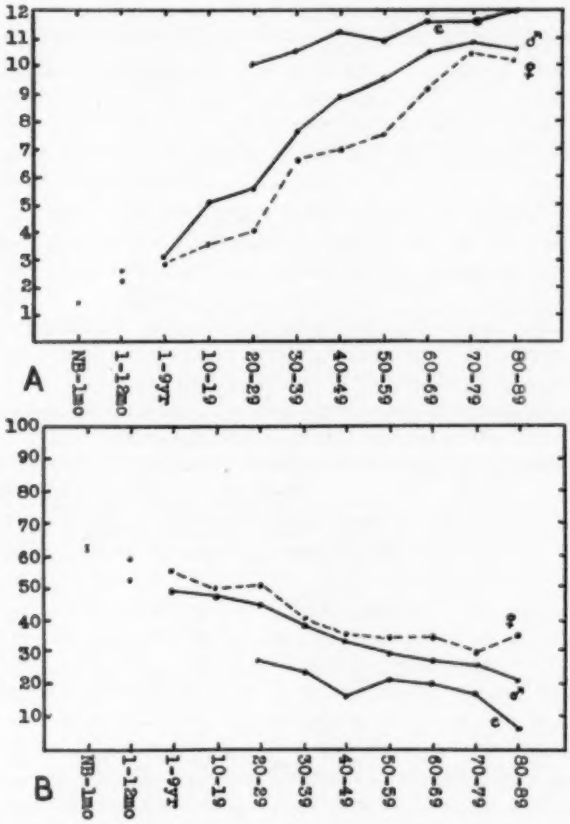

Fig. 8.—*A* graph showing average degree of coronary sclerosis as measured by infiltration of intima (graded 0 to 12) for group with symptoms or death from coronary disease compared with that for whole series divided into males and females. Upper unbroken line, marked *C*, represents coronary-disease group, while broken line represents females of entire series.

B, graph showing relative area of lumen of coronary arteries, as measured in percentage of cross-section area of artery, for group with symptoms or death from coronary disease compared with that for whole series divided into males and females. Lower unbroken line, marked *B*, represents coronary-disease group, while broken line represents females.

Site of Sclerosis.—Although sections for microscopic examination were taken without regard for the distance from the origin of the artery, but only on the basis of the severity of the sclerosis, there was still a remarkable similarity in the location

of the blocks selected (Table 13). Practically all were taken within the first 25% of the length of the artery. This observation agrees with the results of White, Edwards, and Dry [4] and Ackerman, Dry, and Edwards,[13c] who found that the severest sclerosis almost without exception occurred in the proximal third of each artery. There was a slight tendency in the present series for distal progression with age.

TABLE 13.—*Distance from Origin of Artery to Site of Severest Sclerosis in Percentage of Artery Length*

	Average in Males, %	Average in Females, %
NB-1 mo	19	22
1-12 mo	14	19
1-9 yr	17	20
10-19	21	20
20-29	20	22
30-39	23	21
40-49	23	22
50-59	24	23
60-69	25	27
70-79	25	23
80-89	25	24

TABLE 14.—*Comparative Severity of Sclerosis in Right and Left Coronary Arteries, as Percentage of Hearts in Each Age Group*

	Males			Females		
	Right Most Severe, %	Left Most Severe, %	Equal in Right and Left, %	Right Most Severe, %	Left Most Severe, %	Equal in Right and Left, %
NB-1 mo	7	40	53	10	30	60
1-12 mo	16	24	50	0	40	60
1-9 yr	15	25	60	0	25	75
10-19	9	48	43	6	44	50
20-29	7	75	18	5	60	35
30-39	10	45	45	12	60	28
40-49	7	52	41	7	59	34
50-59	9	38	53	4	57	39
60-69	8	21	71	7	43	50
70-79	0	14	86	0	40	60
80-89	7	14	79	22	22	56

A comparison of the severity of sclerosis in the two main coronary arteries was also made (Table 14). This showed that the largest proportion of hearts had either equal severity in the two arteries, or a more severe process in the left coronary artery. Relatively few hearts showed more severe sclerosis in the right.

It must be emphasized that all these findings relate to a group of individuals and not to the disease process as it occurs in a single individual. The process cannot be studied or even detected during life until it reaches a severe stage. However, it may be assumed that the disease process in the individual will generally conform to the average progress of the disease in the general population.

COMMENT

The results obtained from this study indicate that atherosclerosis in the coronary arteries progresses at a nearly uniform rate, beginning in the first decade. The progression appears to continue through the eighth decade, although there is a gradual leveling off in rate, particularly after the fifth decade, according to most of the measurements used. The reduction in area of the lumen showed an almost linear relationship with age.

These results suggest that in most cases age is the most important factor in coronary sclerosis, a finding which confirms the ideas of most previous investigators. The gradual progression of changes from youth to old age is contrary to the generally accepted clinical opinion that coronary disease begins in middle life or later. This gradual progression with age does not suggest that atherosclerosis develops by episodes of rapid increase followed by long periods of quiescence. If episodic development were the case one might expect a more "step-like" appearance of the development at certain periods.

Rather, coronary sclerosis appears to be a continuous process which is inseparable from the "developmental" changes observed in infants and children. The terminal decline in severity is probably due to the survival of resistant individuals into the ninth decade. The number of hearts from persons in this decade which were examined was small, and definite conclusions cannot be drawn. The terminal decline has also been noted by Ackerman, Dry, and Edwards [13c] and White, Edwards, and Dry.[4] The latter authors also attributed the decline to the death of the more severely affected persons and suggested that the disease in the remaining persons might be progressing as before. The slowly progressive nature of the process throws some doubt upon theories which attempt to relate the fundamental cause of atherosclerosis to conditions occurring first in adult life.

The difference in severity of coronary sclerosis between males and females is shown to begin in early childhood, and to continue into senility. It is of sufficient magnitude to place females from 10 to 20 years "behind" males in the development of sclerosis. Once the process is established, however, the rate of progression of coronary sclerosis appears to be nearly the same in both sexes, the difference between the sexes remaining constant or even decreasing in later life. Only measurements of the outside diameter of the coronary arteries show a more rapid rate of increase in men than in women. The beginning of the sex difference before the age of puberty suggests that hormonal differences may not be the fundamental factors. This idea is suggested also by the maintenance of the difference beyond the menopausal age, although in this case an initial difference might continue even though the cause had ceased to be operative. The difference being present throughout life also tends to eliminate the possibility of sociological factors as primary causes. The activities, diets, and responsibilities of children are not much different because of sex.

The sex difference in number of deaths caused by coronary disease is much more striking than the difference seen in comparisons of degree of sclerosis in the two sexes. Comparison of the graphs for individuals dying from or having symptoms of coronary disease with those of the whole group (Fig. 8) showed the line representing the males of the entire series to be much closer to the level required for symptoms than that of the females, and the male line approached this area of symp-

toms earlier in life. It appears that a severe degree of coronary sclerosis is necessary for symptoms or death and that even though the female may have more than 75% as much sclerosis as the male, she may have no clinical signs.

The age at which the sex difference in coronary sclerosis begins is very early. With the thickness of the intima as the criterion, the youngest group which showed a mathematically significant difference was the group 1 to 12 months of age. With relative area of the lumen, the youngest group was that of 1 to 9 years, while with infiltration of the intima, the group 10 through 19 years was the youngest to be significant. However, disregarding the mathematical significance, there was a difference between males and females with every method of measurement down to earliest infancy. It is possible that with a larger group of cases these small differences might also be statistically significant. The work of Dock,[17] Fangman and Hellwig,[10c] and Casarini [10e] on the arteries of infants has suggested the beginning of measurable differences between male and female coronary arteries before birth. This suggestion has yet to be proved. Whether the early thickening of the intima and splitting of the elastic lamina are considered developmental or pathological, they are continuous with the processes of adult life, as are the other changes in the artery. There is no point at which the artery can be said to have stopped growing or developing and started to degenerate or become diseased.

The work of Hamilton [43] has shown that the male of nearly all species studied, including man, has a shorter life span and is more susceptible to most injurious processes than the female, from the time of conception onward. It seems probable that atherosclerosis should also come under this heading, and that the underlying cause of the sex difference lies in this inborn basic difference between the sexes and not in any of the more superficial factors which can be studied. Hamilton attributes the mechanism of the difference to a generally more rapid metabolism in the male.

The effects of hypertension, as measured by cardiac hypertrophy, on the progress of coronary sclerosis were found to be similar to those reported by other investigators.[44] Those hearts which were hypertrophied from hypertension showed remarkably greater degrees of coronary sclerosis in every age group in which they were found than did the nonhypertensive hearts. The difference appeared most marked in the younger groups. This appearance seemed to be due to the fact that the hypertensive hearts showed nearly the maximum measurable degree of sclerosis in almost all the age groups. The difference between hypertensive and nonhypertensive groups was greater than the sex difference. The sex difference persisted in most of the decades among the hypertensive group, although there were not enough cases for significant comparison.

The effect of hypertension on the arteries appears to be basically mechanical,[45] with increased strain on the arterial wall. Wilens [31a] has stated that, while at normal arterial pressure the arterial endothelium is permeable to noncorpuscular elements, the imbibed fluid may be kept in equilibrium. However, with increased arterial pressure there is an increased rate of fluid infiltration and the equilibrium is destroyed, with lipoid and other materials being deposited in the intima.

43. Hamilton, J. B.: The Role of Testicular Secretions as Indicated by the Effects of Castration in Man and by Studies of Pathological Conditions and the Short Lifespan Associated with Maleness, Recent Prog. Hormone Research **3**:257-322, 1948.

44. Master, Dack, and Jaffe.[12] Clawson.[13a] Ackerman, Dry, and Edwards.[13e]

45. Krafka, J., Jr.: The Mechanical Factors in Arteriosclerosis, Arch. Path. **23**:1-19, 1937.

The question of the effect of coronary sclerosis itself on cardiac hypertrophy is beyond the scope of the data presented here. The majority of investigators appear to believe that the hypertrophy is due to a preexisting hypertension.[46] Bell and Clawson,[23] in fact, state that coronary disease prevents further cardiac hypertrophy in hypertension. Others [47] think cardiac hypertrophy is related to coronary sclerosis with cardiac failure, independently of hypertension.

The influence of diabetes on coronary atherosclerosis is well established.[6] Since only 18 cases were included in this series, no conclusions can be drawn, except that individual cases of diabetes frequently show degrees of coronary sclerosis which are completely beyond the usual range in their age groups. Since the primary alteration in diabetes is metabolic, this relationship to atherosclerosis has been influential in promoting the metabolic theories of atherosclerosis, and particularly the incrimination of serum lipids. The elimination of the sex difference in coronary disease among diabetics is interesting and difficult to explain. It supports the idea that serum lipids are somehow different in men and women and cause the sex difference.[48] It also demonstrates that under proper conditions female coronary arteries are as susceptible to atherosclerosis as the male.

The degree of coronary sclerosis existing at death in persons dying with a malignant neoplasm was found to be reduced significantly below that of persons dying without neoplastic disease. In some of the age groups, in middle life, this difference was of the same magnitude as the sex difference. The difference is difficult to explain. There has been no suggestion that arteriosclerosis may affect the occurrence of malignancy or that there is any predisposing condition which might affect both. It is easier to assume that the presence of the tumor influences the course of atherosclerosis.[29c] It is suggested that this influence may be the result of alterations in nutrition during a prolonged terminal illness. Previous investigations have indicated that poor nutrition may affect the course of the atherosclerotic process.[49] Specific studies of the state of nutrition of the persons in the present series were not done because of the lack of sufficient data.

The mechanism through which this change in nutrition might affect the process of atherosclerosis has been thought to be the lowering of the blood lipids during near starvation.[35] This reduction might slow or stop the progress of atherosclerotic lesions. However, since the degree of coronary sclerosis in this group is about 10 years "behind" that of the group without malignancy, it is possible to suppose that there might actually be some regression of atherosclerotic lesions in the human.

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46. Gross, H., and Lisa, J. R.: The Role of Coronary Arteriosclerosis in Cardiac Hypertrophy, New York J. Med. **43**:1030-1032, 1943. Maun, M. E.: The Influence of Coronary Sclerosis, Chronic Congestive Heart Failure and Myocardial Fibrosis on Cardiac Hypertrophy, J. Lab. & Clin. Med. **26**:1239-1248, 1940. Clawson.[13a]

47. Kahn, J. R., and Ingraham, E. S.: Cardiac Hypertrophy and Coronary Arteriosclerosis in Hypertension, Arch. Path. **31**:373-377, 1941. Davis and Blumgart.[42]

48. Block, W. J.; Mann, F. D., and Barker, N. W.: Effect of Small Doses of Heparin in Increasing the Translucence of Plasma During Alimentary Lipemia: Studies in Normal Individuals and Patients with Atherosclerosis, Circulation **4**:674-678, 1951.

49. Malmros, H.: The Relation of Nutrition to Health, Acta med. scandinav., Supp. 246, pp. 137-153, 1950. Ackerman, Dry, and Edwards.[13c] Wilens.[27b] Dublin.[27a]

The comparison of various methods of grading the severity of the atherosclerotic process demonstrated that it makes little difference which is used. The gradually progressive character of the process was shown by any of the methods used. The intima thickens, the lumen is reduced, the elastic lamina degenerates, the artery dilates, and the intima becomes infiltrated with plasma or lipoid, at about the same rate. This observation does not necessarily hold true for the individual case, however, as there is considerable individual variation.

The severity of the lesions in the three main branches of the coronary arteries of each heart was generally found to be of about the same grade. The process in most cases was slightly more advanced in the left main and anterior descending branch, with the right coronary being second, and the left circumflex artery least involved. However, the differences were slight. This finding is similar to that of most investigators.[50]

When death or symptoms from coronary disease had occurred the heart showed severe sclerosis of all three main arteries, no matter at what age the person died. There was an average reduction of the relative area of the lumen to less than 30% of that of the artery. This observation indicates that people must have severe coronary disease before it causes clinical symptoms. Thus, there appears to be a large reserve capacity in the coronary system.

The histological appearance of the atherosclerotic lesion in its well-developed stage is well known. There is still considerable controversy about the earlier stages. Different elements in the structure have been emphasized by various investigators, to the partial exclusion of other changes. Leary [51] has emphasized the macrophages containing lipoid. Altschul [52] has also studied these cells and considers them to be dedifferentiated endothelial cells. Duguid [53] believes that thrombosis causes the appearance of atheroma when the clot becomes organized and degenerated. This opinion has revived an old idea of Rokitansky. Lansing, Alex and Rosenthal [54] have reemphasized changes in the elastic layers as the primary change in atherosclerosis. Many of these changes appear to be taking place at the same time and combining to produce the histological structure seen at the end stage.

SUMMARY

1. The coronary arteries of a series of 536 hearts from autopsies of persons of both sexes from birth to 89 years of age were examined by dissection to determine the severity of atherosclerosis in each.

2. The degree of the sclerosis in each artery was graded on the basis of microscopic examination of the most severely affected area. The degree of infiltration of the intima, relative area of lumen, relative thickness of intima, degree of elastic degeneration, and outside diameter of the artery were graded or measured independently and the results tabulated.

50. Ackerman, Dry, and Edwards.[13c] Clawson.[13a] Ehrich, de la Chapelle, and Cohn.[8c]

51. Leary, T.: The Genesis of Atherosclerosis, Arch. Path. **32**:507-555, 1941.

52. Altschul, R.: Histologic Analysis of Arteriosclerosis, Arch. Path. **38**:305-312, 1944.

53. Duguid, J. B.: Thrombosis as a Factor in the Pathogenesis of Coronary Atherosclerosis, J. Path. & Bact. **58**:207-212, 1946.

54. Lansing, A. I.; Alex, M., and Rosenthal, T. B.: Atheromatosis as a Sequel to Senescent Changes in the Arterial Wall, J. Gerontol. **5**:314-318, 1950.

3. The degree of coronary sclerosis was found to increase progressively with age from early childhood through the seventh decade at a nearly uniform rate.

4. Coronary sclerosis was found to be significantly more severe in males than in females of the same age group even in the first decade of life.

5. The degree of coronary atherosclerosis was found to be much greater in hearts with evidence of hypertension than in nonhypertensive hearts from persons of the same age and sex.

6. Diabetic persons showed severer coronary sclerosis than others, although not enough cases of diabetes were encountered to supply significant data.

7. Persons dying with malignant neoplastic disease were found to have significantly less severe coronary disease than those of the same age dying without malignancy.

8. Death or serious symptoms from coronary sclerosis were found to be generally associated with an advanced degree of sclerosis of all three main coronary-artery branches.

9. A comparison of various methods used to measure the degree of atherosclerosis showed no important differences in the results obtained by each.

DYNAMICS OF INFLAMMATION AND OF REPAIR

III. Effects of Tissue Extracts and of Protein Split Products upon Capillary Permeability and upon Leucocytes

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THE EXACT mechanism by which tissue injury gives rise to acute inflammation has been studied intensively during recent years. Since 1929 Menkin [1] has published many reports and monographs on this subject. He extracted from suppurative exudates a substance which caused increased capillary permeability and migration of leucocytes when injected subcutaneously. Later he found in tryptic digests of serum and in testicular tissue a substance having similar effects. He believed that this was a chemical entity "which appears as if it may belong to the group of relatively simple polypeptides." He named this substance leucotaxine and assigned to it the chief role in the dynamics of inflammation.

These findings attracted attention to products of protein cleavage as factors initiating inflammatory reactions. Duthie and Chain [2] found that a substance which increased permeability was present in digests of several proteins (serum albumin, globulin, fibrin, egg albumen, etc.) obtained by the action of pepsin, trypsin, or papain. They noted that the reactions were stronger after partial digestion and weaker if the period of digestion was prolonged. The products of peptic digestion of fibrin, purified by adsorption on fuller's earth, regularly caused increased capillary permeability, as shown by dye tests. Histologic sections of injected tissues showed leucocytic infiltrations. When tested with leucocytes in vitro, these polypeptides had chemotactic effects. Duthie and Chain were unable to confirm Menkin's claim that leucotaxine was a single substance. They found that various polypeptides resulting from the action of proteolytic enzymes on animal proteins have all the properties ascribed to leucotaxine.

Cullumbine and Rydon [3] extracted leucotaxine from several sources: suppurative exudates, blister fluid, pulmonary edema fluid, peptic digestion of fibrin, and succus entericus. The effects of the latter were attributed to the digestion of the mucosa

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1. Menkin, V.: Studies on Inflammation: Fixation of Vital Dyes in Inflamed Areas, J. Exper. Med. **50**:171, 1929; Dynamics of Inflammation, New York, The Macmillan Company, 1940; Newer Concepts of Inflammation, Springfield, Ill., Charles C Thomas, Publisher, 1950.

2. Duthie, E. S., and Chain, E.: A Polypeptide Responsible for Some of the Phenomena of Acute Inflammation, Brit. J. Exper. Path. **20**:417, 1939.

3. Cullumbine, H., and Rydon, H. N.: A Study of the Formation, Properties and Partial Purification of Leukotaxine, Brit. J. Exper. Path. **27**:33, 1946.

by enzymes of the intestinal secretions. They believed that leucotaxine was produced from tissue proteins by the action of a proteinase either liberated from the tissues by injury or enabled to act by the inhibition of some antienzyme normally present in the tissues. They defined leucotaxine as "a substance or group of closely related substances having the common property of inducing a local increase of capillary permeability." The inclusiveness of this definition lowers its usefulness; histamine, snake venom, peptones, and other unrelated substances would fill its requirements.

Spector [4] questioned whether the effects reported by Menkin and by Cullumbine and Rydon were due to a single entity or were produced by many substances. Tryptic and peptic digests of fibrin were made, fractionated, and purified by standard chemical methods. Each fraction so derived was tested for three effects: capillary permeability as shown by the dye test, migration of leucocytes seen by histologic examination of the lesions, and endothelial swelling seen in the lesions. The active principle of all such digests was found to be the peptide fraction. Each fraction was subjected to analysis to determine its chemical structure, especially the number of amino acids in the peptide chain. He found that polypeptides with 8 to 14 amino acids per chain showed the three biological properties mentioned. On the other hand, polypeptides with an average chain length of five amino acids produce only the last two features; capillary permeability was not increased. Destruction of the peptide linkage by acid hydrolysis destroyed its activity and at the same time liberated its constituent amino acids. These findings substantiate the observation by Duthie and Chain that products of prolonged digestion gave weaker reactions.

Spector demonstrated that a pancreatic trypsin inhibitor, a pure crystalline peptide with a molecular weight of about 6,000, produced each of the effects mentioned above. He concluded "that such properties are possessed not by one but by many peptides within certain limits of molecular size, and that the biological effect may vary with the length of the peptide chain." He also concluded that the conception of leucotaxine as a single entity can no longer be entertained.

In a previous report we [5] showed that watery extracts of various animal tissues will produce local hyperemia, edema, and leucocytic infiltration when injected intracutaneously. These phenomena were shown by histologic examination of stained sections of the lesions. The effects of such extracts upon capillary permeability are of interest since leucocytic infiltration is an early and important feature of acute inflammation.

Various colloidal dyes, if injected intravenously, will escape into the tissues in any area where capillary permeability is increased. This fact was utilized by the workers cited above and by others in studies on inflammation. Two series of tests were made: one upon extracts of normal tissues in which opportunity for autolysis had been allowed, and one upon various commercial products of protein cleavage. The extracts of tissues and the commercial products of protein cleavage were tested by the same method, usually simultaneously in the same animal. Another purpose of this project was to observe leucocytic infiltration and to note any correlation between it and the degree of capillary permeability in areas where the various substances were injected.

4. Spector, W. G.: The Role of Some Higher Peptides in Inflammation, J. Path. & Bact. **63**:93, 1951.

5. Moon, V. H., and Tershakovec, G. A.: Dynamics of Inflammation and of Repair: The Trigger Mechanism of Acute Inflammation, A. M. A. Arch. Path. **52**:369, 1951.

METHODS

Tissue Extracts.—A weighed quantity of each tissue was suspended finely in 4 volumes of 0.9% NaCl solution by means of a Waring Blendor. After autolysis at room temperature for two to three hours, the suspension was centrifuged; the supernatant fluid was adjusted to pH 7, clarified by centrifugation at high speed, and sterilized by means of a Seitz filter. Extracts of lung, spleen, kidney, muscle, and liver were made as described and were kept under refrigeration until used. Most of the tissues were from rabbits; a few extracts of dog tissue were tested for comparison, but the results were not notably different in any instance.

Products of Protein Cleavage.—Various commercial products were used as test substances without chemical treatment or purification. These included peptone from meat (Eimer & Amend); beef extract, N.F. (Wilson), and Neopeptone, bactoprotone, proteose peptone, and Bacto-Peptone (each by Difco). We realize that these substances are of unknown chemical composition; in fact, that is why they were chosen. They each contained products derived from animal proteins, and, as shown by our tests, each of them caused varying degrees of capillary

TABLE 1.—*Capillary Permeability Induced by Extracts of Normal Tissues*

Extracts	Tests	Strong	Moderate	Slight	Doubtful	Negative
Kidney						
Undiluted	10	6	4	0	0	0
Diluted 1:2	8	2	5	1	0	0
Lung						
Undiluted	7	5	2	0	0	0
Diluted 1:2	4	0	4	0	0	0
Spleen						
Undiluted	9	5	4	0	0	0
Diluted 1:2	9	3	4	2	0	0
Liver						
Undiluted	11	3	5	3	0	0
Diluted 1:2	4	0	4	0	0	0
Muscle						
Undiluted	17	0	0	0	3	14
Treated	10	1	4	5	0	0

permeability and exhibited chemotactic properties. Hence these commercial products conform to proposed definitions for leucotaxine. These substances were dissolved in 0.9% NaCl solution, adjusted to pH 7, centrifuged if necessary for clearing, sterilized by filtration (Seitz), and kept under refrigeration until used. The usual concentration of these solutions was 2%. When such a solution gave a weak reaction, a 4% solution was tested.

A peptic digest of fibrin was prepared as follows: Five grams of fibrin (Nutritional Biochemical Corporation) was suspended in 100 ml. of H_2O and acidified with 5 N HCl to pH 2. Five hundred milligrams of pepsin (1:10,000, National Biochemical Corporation) was dissolved in a few milliliters 0.1 N HCl and added to the fibrin suspension. The digestion mixture was incubated in a water bath at 37 C. for two hours. During incubation the reaction was maintained at pH 2. After incubation the digest was neutralized to pH 7, deproteinized by boiling for five minutes, centrifuged, and filtered. This digest contained 5 gm. of dry residue in 100 ml.

Healthy albino rabbits weighing about 3 kg. were used. Since individual animals vary somewhat in their responses, each substance was tested on several animals. The fur on the abdominal area was cut closely with an electric clipper, taking care not to injure the skin. A freshly prepared 1% solution of trypan blue in 0.9% NaCl solution was injected into the marginal ear vein, 1 ml. per kilogram of body weight. Then 0.2 ml. of the solution to be tested was injected into the skin with a 27-guage needle. As controls, 0.9% NaCl solution was injected as described. The reactions to the injections began almost immediately and reached their maximum in 15 to 30 minutes, at which time readings were made and photographs taken.

Each of the preparations mentioned was tested in a series of increasing dilutions to determine the lowest concentration that would cause increased capillary permeability. Rabbits were killed at varying intervals (one to three hours) after the injections, and the lesions were prepared for histologic examination.

RESULTS

Increased capillary permeability was shown by an area of deep blue surrounding the point of injection. Variations in the density of the blue color and in the size of the area indicated variations in the degree of the effect. These degrees were recorded as strong, moderate, slight, doubtful, and negative. The results of these tests on tissue extracts are given in tabular form.

A marked difference is noted in the results from different tissue extracts. The strongest reactions were obtained from extracts of kidney, lung, and spleen; liver extract caused moderate reactions, while muscle extract prepared as described above produced little more evidence of capillary permeability than did isotonic sodium chloride solution (Figs. 1 and 2).

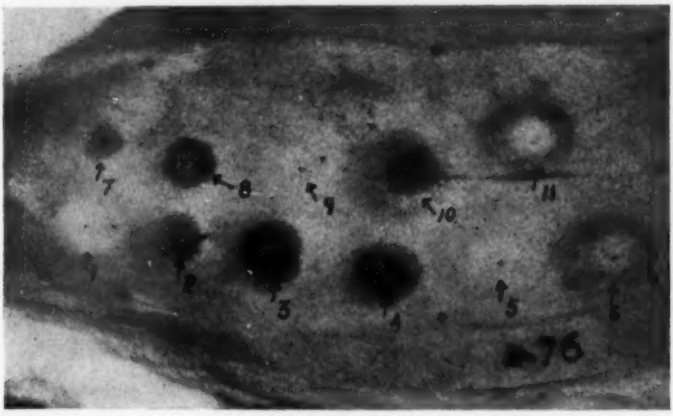

Fig. 1.—Rabbit No. 76 showing capillary permeability produced by (1) NaCl, control; (2) extract of kidney; (3) extract of lung; (4) extract of spleen; (5) extract of muscle; (6 and 11) histamine, 0.1%, ring reactions; (7) extract of liver; (8) Neopeptone, 2%; (9) meat peptone, 2%; (10) bactoprotone, 2%.

Another extract of muscle was prepared as follows: Finely cut muscle tissue was beaten into a suspension with 4 volumes of cold 0.9% NaCl solution (0 to 5 C.) by means of a Waring Blendor, kept under refrigeration, then centrifuged at low temperature. The supernatant fluid was acidified with diluted HCl to about pH 4. After the addition of $FeSO_4$ to concentration M 0.004, the muscle extract was incubated at 35 C. for four to six hours. The reaction was then adjusted to pH 7, and the fluid was treated as described for extracts of tissues. Similar procedures were found favorable for proteolysis of striated rabbit muscle by Snoke and Neurath.[6] This autolysate produced moderate permeability of capillaries when tested (see muscle, treated, in Table 1). These tissue extracts provoked positive trypan blue reactions in higher dilutions as follows: kidney extract, dilution 1:256,

6. Snoke, J. E., and Neurath, H.: The Proteolytic Activity of Striated Rabbit Muscle, J. Biol. Chem. **187**:127, 1950.

slight reaction; spleen extract, dilution 1:64, moderate reaction; lung extract, dilution 1:32, slight reaction; liver extract, dilution 1:8, slight reaction. In higher dilutions than these the reactions were negative.

The results of tests on various commercial preparations which contain products of protein cleavage and on our own peptic digest of fibrin are tabulated below. With these are shown the effects of histamine phosphate for comparison and the 49 control injections for both series.

It is noted that Neopeptone, bactoprotone, and proteose peptone produced strong reactions, while the two remaining peptone preparations and beef extract were definitely weaker in their effects. Peptic digest of fibrin behaved similarly to the other peptone preparations, producing a strong reaction in 1:5 and 1:10 dilutions (1% and 0.5% respectively). Bacto-Peptone affected capillary permeability slightly more than peptone from meat. Control injections with 0.9% NaCl solution were uniformly negative with occasional minute accumulations of the dye

Fig. 2.—Rabbit No. 81 showing capillary permeability produced by (1) NaCl, control; (2) Bacto-Peptone, 4%; (3) meat peptone, 4%; (4) Neopeptone, 2%; (5) proteose peptone, 2%; (6) bactoprotone, 2%; (7) beef extract, 4%; (8) Bacto-Peptone, 4%. X indicates rudimentary mammary nipples.

at the site of the needle prick. Tests with histamine phosphate showed two types of trypan blue reaction: In lower concentration (0.01% solution) it produced a dense blue homogeneous discoid patch, while 0.1% solution produced a blue ring surrounding a pale area. Similar variations in the reactions to histamine were observed by Rocha e Silva and Dragstedt[7] on rabbits and by Spector on rats.

This phenomenon may be explained as follows: The stronger solution of histamine causes arteriolar constriction sufficient to produce ischemia in the central area, hence the pale area surrounded by a ring. The weaker solution does not cause arteriolar contraction but only capillary permeability, hence the homogeneous discoid area of coloration. We have noted that, with the passage of time, the ring reaction became discoid as the arteriolar effect subsided. We found similar vari-

7. Rocha e Silva, M., and Dragstedt, C. A.: The Capillary Permeability Test in Rabbits, J. Pharm. & Exper. Therap. **73**:405, 1941.

ations in tests of strong and weak solutions of peptic digests of fibrin, and Spector made the same observation in testing peptide preparations. These variations seem to be quantitative rather than qualitative and should not be regarded as differential characteristics, as some have implied.

Dilutions ranging from 0.5 to 0.001% were tested to determine how high a dilution of these products would evoke a positive reaction. Briefly stated, the results were as follows: Positive reactions were produced by meat peptone (2%), by beef extract (0.5%), by Neopeptone (0.01%), by bactoprotone (0.01%), by proteose peptone (0.01%), by Bacto-Peptone (0.5%), and by peptic digest of fibrin (0.01%). In lower concentrations than these the reactions were doubtful or negative. These results indicate that the amount or the potency of substances producing capillary permeability varied widely in the different preparations tested, including the extracts of tissues.

TABLE 2.—*Capillary Permeability Induced by Products of Protein Cleavage*

Solutions	Tests	Strong	Moderate	Slight	Doubtful	Negative
Meat peptone 4%	10	0	3	6	1	0
Meat peptone 2%	10	0	2	3	3	2
Bacto-Peptone 4%	10	0	7	2	1	0
Bacto-Peptone 2%	10	0	2	7	1	0
Beef extract 4%	6	0	2	4	0	0
Beef extract 2%	9	0	6	0	2	1
Neopeptone 2%	8	5	3	0	0	0
Neopeptone 1%	10	9	1	0	0	0
Bactoprotone 2%	9	9	0	0	0	0
Bactoprotone 1%	6	5	1	0	0	0
Proteose peptone 2%	10	8	2	0	0	0
Proteose peptone 1%	3	2	1	0	0	0
Digest of fibrin 0.5%	7	7	0	0	0	0
Histamine 0.01%	6	3	3	0	0	0
NaCl 0.9%	49	0	0	0	17*	32

* Accumulation of dye only in the site of the needle prick.

LEUCOCYTIC INFILTRATION

A representative number of reactions, both positive and negative to the trypan blue test, were examined histologically. These included each of the extracts of tissues, each of the commercial products of protein digestion, histamine, and controls. Animals were killed at intervals of two or three hours after the injections were made. The findings in these lesions are both interesting and significant.

The control lesions made by injection of 0.9% NaCl solution showed no infiltration of leucocytes in most instances. However, in three out of seven sections there was a slight infiltration. This may be interpreted as due to injury of connective tissue resulting from mechanical distention by the injected fluid. This factor was kept in mind in the interpretation of other sections. Lesions resulting from injection of histamine phosphate, 0.01%, were similar to those of the controls.

Lesions produced by injections of extracts of kidney, spleen, lung, and muscle (Fig. 3) contained moderate to marked leucocyte infiltration. This corroborates our findings in a previous report.[5]

Lesions produced by injection of meat peptone, 4% solution, contained moderate to marked leucocytic infiltration. In lower concentrations leucocytic infiltration was only slight. Beef extract, 4%, produced a strong infiltration with leucocytes (Fig. 4*B*). Proteose peptone, 2% solution, regularly produced dense infiltration with leucocytes, while Neopeptone and bactoprotone produced only moderate infiltration. Peptic digest of fibrin, 0.5% solution, caused marked leucocytic infiltration (Fig. 4*A*). Bacto-Peptone, 4%, produced slight to moderate infiltration.

There is lack of correlation between the leucocytic infiltration seen in these sections and the degree of capillary permeability shown by the tests with trypan blue. For example, histamine produced distinct capillary permeability but no leucocytic infiltration, while extracts of muscle and beef cause only slight perme-

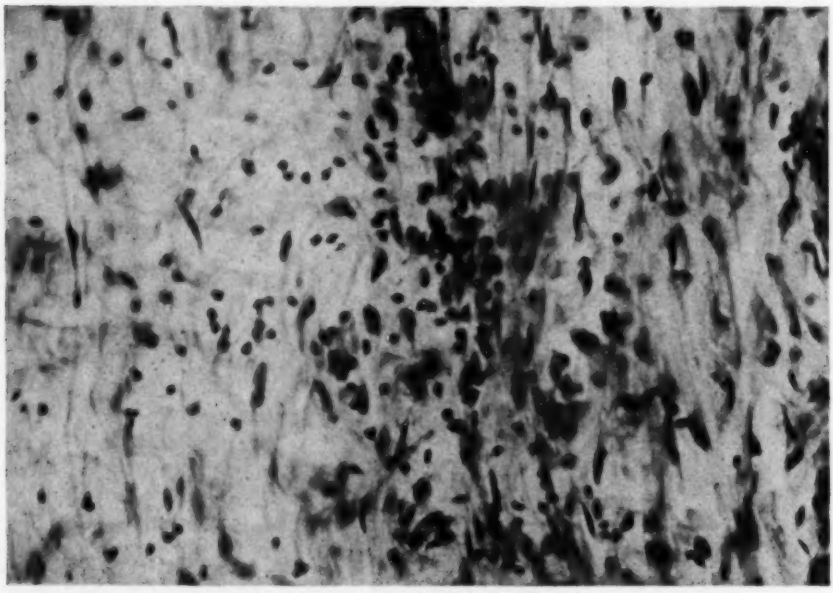

Fig. 3.—Photomicrograph of lesion made by injecting extract of rabbit muscle. Moderate leucocytic infiltration and edema are seen; × 220.

ability but dense infiltration. In other instances—using bactoprotone, proteose peptone, and fibrin digest—both capillary permeability and infiltration with leucocytes were marked.

Perhaps these variations may be explained on the basis of Spector's observation that the polypeptides having long amino acid chains caused both capillary permeability and leucocytic migration. Those with short chains (those in which digestion or hydrolysis was more advanced) failed to increase the permeability of endothelium but caused leucocytic infiltration. It is possible that the meat peptone, extract of beef and muscle, contained only the simpler polypeptides resulting from advanced digestion and hence did not increase the permeability of the capillaries. On the other hand, the protein cleavage products (such as Neopeptone, proteose

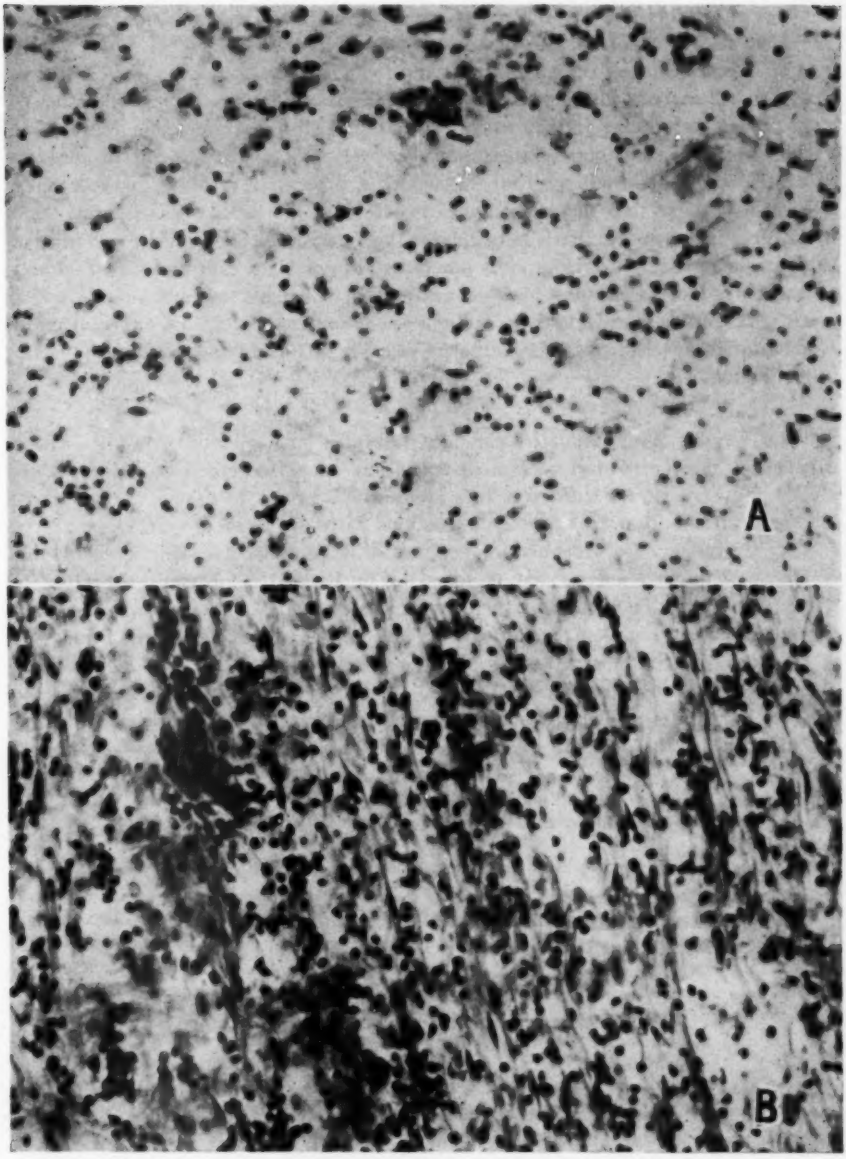

Fig. 4.—*A*, photomicrograph of area injected with digest of fibrin. Severe edema and leucocytic infiltration are shown; × 220. *B*, photomicrograph of area injected with beef extract, showing intense leucocytic infiltration and moderate edema; × 220.

peptone, and digest of fibrin) and the extracts of lung, kidney, and spleen contained more of the larger peptide molecules which result from less advanced digestion. We have lacked chemical facilities to test this hypothesis.

Our results confirm those of others who found that various products of protein cleavage will increase the permeability of capillaries and cause migration of leucocytes when introduced intradermally. We found also that aqueous extracts of various normal tissues possess these same properties. This may be due to products of protein cleavage resulting from the action of proteinase residing in the tissues. It is also possible that the unchanged cytoplasm of the cells, released from them by injury, increases capillary permeability and attracts leucocytes to the injured area, as suggested by Lewis [8] and by one of us (Moon [9]). Duthie and Chain found a permeability-increasing substances in extracts of liver, kidney, and muscle which were with and without autolysis. It will be difficult to determine experimentally which of these mechanisms is operative or whether each is a factor in initiating acute inflammation.

CONCLUSIONS

The weight of accumulated evidence indicates that no one substance but a number of substances derived from tissues may cause increased capillary permeability and may attract leucocytes into areas where injury to tissues has occurred. These features appear to be general reactions to products of protein cleavage.

These products are probably polypeptides of large molecular size. They include products of tissue autolysis and perhaps the unchanged cytoplasmic substance of the injured cells. The action of such substances is of high importance in initiating the inflammatory reaction promptly when and where tissues have been injured.

Various protein derivatives of unknown chemical structure and composition elicit the same responses when injected as those claimed for leucotaxine, the chemical structure of which is indeterminate. This fact lessens the probability that the initial reactions of acute inflammation are due to one and the same chemical entity.

8. Lewis, T.: Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, Ltd., 1927.

9. Moon, V. H.: Mechanism of Acute Inflammation, Arch. Path. **20**:561, 1935.

COMPARISON OF PATHOLOGIC EFFECTS OF RADIATION IN WEANLING AND ADULT RATS

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ROCHESTER, N. Y.

INTRODUCTION

IT HAS been observed in this laboratory (Noonan [1]) that rats 4 weeks of age (weanlings) have a mean survival time of approximately 3 days, as compared with approximately 10 days for adult rats, after a single dose of 800 r (250 kv. unfiltered) total-body x-irradiation. It was of interest, therefore, to investigate the possible differences in the pathologic picture in weanling and adult rats receiving 800 r of unfiltered x-irradiation in order to determine whether or not the difference in survival time can be explained on the basis of differences in degree of histopathologic damage of one or more tissues and whether or not differences in degree of pathologic change can be explained by differences in the amount of unfiltered radiation penetrating to the radiosensitive tissues of rats of such unequal size.

MATERIALS AND METHODS

Animals.—Fourteen adult male rats, weighing from 320 to 458 gm. (average 380 gm.), and 12 weanling male rats, 28 days of age and weighing from 52 to 64 gm. (average 58.0 gm.), were subjected in two separate groups, respectively, to a single total-body dose of 800 r of x-rays. Two male adult and two male weanling rats were used for control tissues and were not irradiated. The rats were of the Wistar strain. They were housed singly in separate cages and were given Purina Fox Chow (with meat meal) and water ad libitum, the water being supplied in inverted glass cylinders suitable for observing approximately the degree of water intake.

Dosimetry.—The x-rays were unfiltered and were obtained by means of a Picker Industrial 250 kvp generator having a half value layer of 0.34 mm. Cu. The dose of 800 r was given at the rate of 85 r per minute and was measured in air by means of Victoreen r-meters placed approximately 0.5 in. (1.27 cm.) above the treatment table or approximately at the level of the anterior abdominal region of the normally crouching animal. The distance from the skin of the back to the target overhead was 23 in. (58.42 cm.).

It is to be expected that the greater thickness of the skin, muscles, bones, and other tissues of adult rats would reduce the amount of such unfiltered radiation to penetrate to internal sensitive tissues more greatly in adult rats than in weanling rats and that irradiated weanling rats would exhibit greater pathologic changes as a result. In order to obtain some indication of the magnitude of the maximum difference in internal dose, a comparison was made between readings obtained on Victoreen r-meters placed at the same distance from the target, some in air, and some with the sensitive ends of the meters (protected from moisture by rubber finger cots) just inside the anterior abdominal wall of an adult and weanling rat. The thickness of rat body

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This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project.

1. Noonan, T. R.: Personal communication to the author.

between the center of the chamber and the dorsal surface of the rat was approximately 0.75 in. (1.91 cm.) for the weanling rat and 1.25 in. (4.45 cm.) for the adult rat. The chambers placed intra-abdominally were covered inferiorly only by the anterior abdominal wall. Experimental conditions were simulated. The abdominal measurements in adult and weanling rats were 74% and 86%, respectively, of the measurements in air. In terms of 800 r measured in air, the anterior abdominal measurements would be approximately 592 r and 688 r for adults and weanlings, respectively, representing a difference of approximately 100 r.

The physical inaccuracies of these direct dosimetric comparison procedures are recognized. However, since highly refined dosimetric experiments are not within the scope of this paper, the difference in the anterior abdominal measurements between adult and weanling rats is corrected by the addition of 50% of the difference and assumed to be 150 r for the sake of the discussion of pathologic changes and in order that errors in dosimetric procedure may be accounted for. It seems reasonable that the maximum difference in internal dose between the adult and weanling rats is within 150 r for a dose of 800 r measured in air under the conditions of this experiment. It is possible with a dose of 800 r measured in air that the dose at some sites within the adult rat body may be as low as 500 to 530 r, while at similar sites in the weanling body, only as low as 650 to 680 r.

Sacrifice Schedule.—Two irradiated adult rats were sacrificed by ether inhalation on the 1st, 2d, 3rd, 4th, 6th, 8th, and 10th days after irradiation. Two irradiated weanlings were sacrificed on the first, second, and third days after irradiation, and one was sacrificed on the fourth day. The remaining five weanlings died during the night between the third and fourth days. No spontaneous deaths occurred in the adult group. The control rats were sacrificed immediately after the last sacrifice in each respective group.

Samples of radiosensitive organs taken routinely at autopsy for histopathologic examination included submaxillary lymph nodes, abdominal (cecal) lymph nodes, spleen, liver, testes, stomach, duodenum, jejunum, ileum, colon, and femur with bone marrow intact. Samples were taken of all grossly observed abnormalities. The samples were fixed in Bouin's fluid, sectioned at a thickness of approximately 7 μ, and stained with Harris' hematoxylin and eosin. Additional samples of liver were fixed in an alcohol-formalin solution and were stained for glycogen with Best's carmine stain. Additional samples of liver were fixed in 10% neutral formalin solution and stained for neutral fat with Sudan IV.

Blood cell counts, hemoglobin determinations, and body weights were obtained on all rats before irradiation and on all survivors on the 1st, 2d, 3d, 4th, 6th, 8th, and 10th days after irradiation.

OBSERVATIONS

Clinical Observations.—The weanling rats ingested very little food and water during the entire period of the experiment. They were lethargic immediately after irradiation and prostrate during the second and third days, exhibiting severe diarrhea.

The adult rats ingested considerable amounts of food every day but the second and third days after irradiation, when their food intake appeared markedly reduced. They ingested water every day in approximately normal amounts, however. Adult rats exhibited signs of diarrhea during the second and third days, but this was moderate as compared with the severe diarrhea of the weanling rats.

Body Weights.—In Table 1 are presented the body weights of irradiated adult and weanling rats according to days after irradiation, and in Table 2 and in Chart 1 are presented the average weight losses for irradiated adult and weanling rats according to days after irradiation, expressed in terms of the per cent of original weight loss.

From Chart 1 it can be seen that the average per cent weight loss for adult rats rose gradually after irradiation to reach the level of 19.9% by the 10th day.

TABLE 1.—*Body Weights of Adult and Weanling Rats Receiving a Single Dose (800 r) of X-Rays*

Rat No.*	Preirradiation Weight, Gm.	Weight, Gm., Days After Irradiation						
		1	2	3	4	6	8	10
A 1	442	411	...	...	...	...	...	...
A 2	320	300	...	...	...	...	...	...
A 3	408	390	386	...	...	...	...	...
A 4	410	400	396	...	...	...	...	...
A 5	390	374	362	362	...	...	...	...
A 6	370	356	346	336	...	...	...	...
A 8	406	392	390	366	362	...	...	...
A 9	364	348	338	328	330	322	...	...
A 10	370	352	346	334	332	317	...	...
A 11	458	428	422	428	422	416	402	...
A 12	364	310	302	290	284	284	288	285
A 13	334	318	310	278	286	242	234	...
A 14	360	348	338	318	314	302	312	295
A 15	330	308	278	280	268	...	...	...
Average	380.4	359.6	351.1	332.0	324.7	313.8	309.0	290.0
W 17	56	56	..	..	..	..	..	..
W 18	54	54	..	..	..	..	..	..
W 19	63	62	58	..	..	..	..	..
W 20	58	58	50	..	..	..	..	..
W 21	54	54	44	40	..	..	..	..
W 22	58	58	52	44	..	..	..	..
W 23	60	60	52	44†	..	..	..	..
W 24	59	60	52	54	52	..	..	..
W 25	52	56	48	40	40†	..	..	..
W 26	64	62	56	53†	..	..	..	..
W 27	56	54	52	43†	..	..	..	..
W 28	62	62	54	50	46†	..	..	..
Average	58.0	58.0	51.8	46.0	46.0	..	..	..

* A, adult rat; W, weanling rat.
† Weight at death.

TABLE 2.—*Per Cent of Original Weight Lost in Adult and Weanling Rats Receiving a Single Dose (800 r) of X-Rays*

Rat No.*	Preirradiation Weight, Gm.	% Weight Lost, Days After Irradiation						
		1	2	3	4	6	8	10
A 1	442	7.0	..	..	..	..	..	..
A 2	320	6.2	..	..	..	..	..	..
A 3	408	4.4	5.4	..	..	..	..	..
A 4	410	2.4	3.4	..	..	..	..	..
A 5	390	4.1	7.1	7.2	..	..	..	..
A 6	370	3.7	6.5	9.2	..	..	..	..
A 8	406	3.4	3.9	9.9	10.8	..	..	..
A 9	364	4.4	7.1	9.9	9.3	11.5	..	..
A 10	370	4.8	6.5	9.7	10.3	14.3	..	..
A 11	458	6.5	7.8	6.5	7.8	9.2	12.2	..
A 12	364	14.8	17.0	20.3	22.0	22.0	20.9	21.7
A 13	334	4.8	7.2	16.7	14.4	27.5	30.0	..
A 14	360	3.3	6.1	11.6	11.8	16.1	13.3	18.1
A 15	330	6.6	15.7	15.7	18.8	..	..	..
Average	380.4	5.45	7.8	11.6	13.15	16.7	19.1	19.9
W 17	56	0.0	..	..	..	..	..	..
W 18	54	0.0	..	..	..	..	..	..
W 19	63	1.6	8.0	..	..	..	..	..
W 20	58	0.0	13.8	..	..	..	..	..
W 21	54	0.0	18.5	26.0	..	..	..	..
W 22	58	0.0	10.3	24.1	..	..	..	..
W 23	60	0.0	13.3	26.6†	..	..	..	..
W 24	59	1.7‡	11.8	8.5	11.8	..	..	..
W 25	52	7.7‡	7.7	23.0	23.0†	..	..	.
W 26	64	3.1	12.5	17.2†	..	..	..	..
W 27	56	3.6	7.1	23.2†	..	..	..	..
W 28	62	0.0	13.0	19.4	25.8†	..	..	..
Average	58.0	0.09‡	11.6	21.0	20.2	..	..	..

* A, adult rat; W, weanling rat.
† Weight at death.
‡ Per cent of weight gained.

In the case of the rapidly growing weanling rats, some showed a weight gain at the first post-irradiation day, while others lost weight or remained at the same weight, so that the average value indicates essentially no weight change at this time. At the second and third days, however, there was a precipitous loss of weight, reaching the level of 21% by the third day.

Microscopic Observations.—Liver: The liver revealed no destruction of cells in either adult or weanling rats. Neutral fat was increased slightly in the adult liver on the 1st and 2d days and to a fairly marked degree on the 3d and 4th days,

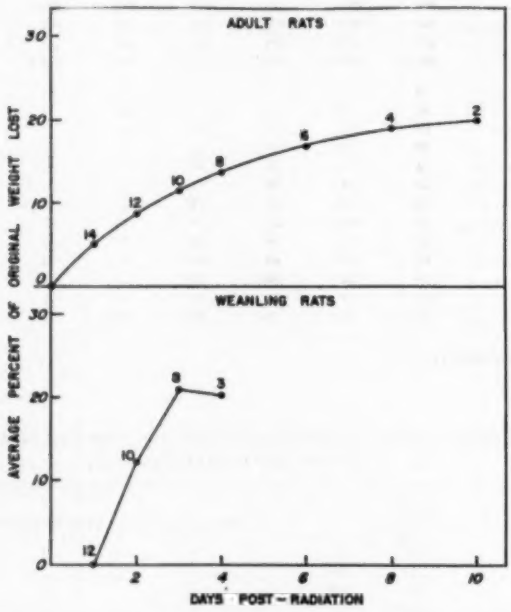

Chart 1.—Average per cent of original weight lost in adult and weanling rats receiving a single dose (800 r) of x-rays. Number of rats indicated at each point.

decreasing thereafter to the normal condition of little or no neutral fat by the 10th day. Weanling rats revealed no increase of neutral fat in the liver.

Marked reductions in the glycogen content of the liver were found on the third and fourth days in weanling rats and on the fourth and sixth days in adults. The portal regions of the hepatic lobules were those principally involved in the loss of glycogen.

Gastrointestinal Tract: 1. Intestines. Following irradiation, in both weanling and adult rats there was early (first day) marked depression of mitotic activity in the intestinal mucosa and swelling and distortion of basal gland cells, followed (second day) by frank degeneration, necrosis, and desquamation of epithelial cells; formation of "cystic" glands containing cellular debris, and disorganization of the mucosal pattern, with decrease of amount of epithelium and relative increase of connective tissue.

In general, the intestinal damage of weanling rats was considerably greater than that of adult rats. Intestinal changes reached a maximum degree on the third day. The intestinal mucosa of the five weanling rats which died between the third and fourth day sacrifice periods revealed marked damage, greater than that observed in sacrificed weanlings. The intestinal mucosa of the weanling rats sacrificed on the fourth day revealed considerable regenerative activity and recovery of epithelium. Regeneration was in progress in the adult rats on the third day and was essentially complete on the fourth day and thereafter.

In general, the proximal portions of the intestinal tract, the duodenum and the jejunum, suffered greater damage than the more distal portions, the ileum and the colon.

Gastorintestinal Tract: 2. There were no marked and consistent histopathologic changes in the stomach. In both adult and weanling rats, mitotic activity was depressed for two days, and small numbers of zymogenic cells became degenerate.

Testes: The testes of adult and weanling control rats were normal, although the testes of weanling rats presented evidence of immaturity, such as lack of spermatozoa, subnormal numbers of spermatids, and small seminiferous tubules. Spermatogonia and spermatocytes were present in full complement for the size of the tubules in weanling testes, however.

There was rapid degeneration and decrease of spermatogonia in weanling testes following irradiation, these cells being markedly reduced by the second day. In adults a comparable stage was reached by the third day. In the weanling testes there were also moderate reductions of spermatocytes, and many of the spermatocytes remaining were degenerate. In adult testes spermatocytes were not reduced, and only a few became degenerate. On the third and fourth days, degenerate spermatids were numerous in weanling testes but were rare at all times in adult testes.

Hematopoietic Organs: 1. Lymph nodes. In weanling lymph nodes, depletion of lymphocytes was rapid, and atrophy of the nodes was almost extreme by the 24th hour after irradiation. There was no evidence of regeneration of lymphocytes thereafter. Loss of lymphocytes was never more than moderate in adult rats, and repopulation of the nodes with lymphocytes was in evidence on the sixth day and thereafter. Hemorrhage was observed in lymph nodes of many of the adult rats sacrificed after the fourth day, however.

Hematopoietic Organs: 2. In addition to normal amounts of lymphatic tissue, the spleens of control weanling rats contained fairly large amounts of extramedullary hematopoietic tissue containing both erythropoietic and myelocytopoietic cells. Extramedullary hematopoiesis was only slightly in evidence in the spleens of adult control rats.

In weanling spleens, depletion of lymphocytes progressed rapidly and became almost extreme by the third day after irradiation. No regeneration of lymphocytes was evident on the fourth day. In adult spleens, lymphatic hypoplasia was never more than moderate, and there was some indication of regeneration of lymphocytes on the 6th, 8th, and 10th days.

In weanling spleens, extramedullary hematopoiesis disappeared completely in all cases. The traces of extramedullary hematopoietic tissue in adult spleens disappeared also. On the 8th day, however, such tissue returned in considerable

amounts in the adult spleens, and on the 10th day it was fairly abundant. Megakaryocytes were markedly reduced in number in irradiated adult and weanling spleens throughout the experiment.

Hematopoietic Organs: 3. Depletion of the femoral bone marrow of irradiated weanling rats progressed rapidly, becoming almost extreme by the third day after irradiation, while in adults this stage was not reached until the sixth day after irradiation. The weanling marrow revealed marked congestion and considerable hemorrhage, while in the adult marrow these changes were only slight. No recovery was observed in weanling marrow on the fourth day. In adults, however, there was considerable regeneration of marrow cells on the 8th and 10th days, the

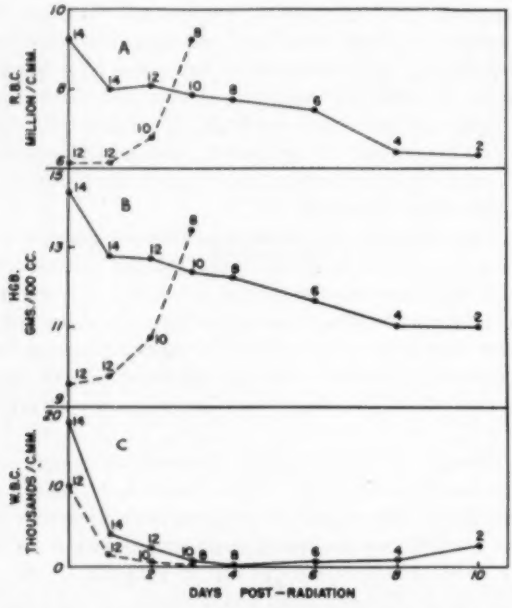

Chart 2.—Average hematologic values for adult (solid line) and weanling (broken line) rats receiving a single dose (800 r) of x-rays Number of rats indicated at each point.

intervals at which supranormal extramedullary hematopoiesis was evident in the adult spleens.

Marrow depletion was earlier and greater at the growing ends of the femora than in the shaft, and regeneration began in the shaft.

Circulating Blood Cells: Presented in Table 3 and in Chart 2 are the important average hematologic values obtained from the experimental animals.

Circulating Blood Cells: 1. Erythrocytes and hemoglobin. Fom Chart 2 *A* and *B* one can see that the erythrocyte count and hemoglobin content of the blood of adult rats dropped to 86% and 89%, respectively, of original values by the 24th hour after irradiation and then declined slowly and gradually from the 2d day to the 10th day after irradiation, when the erythrocyte count and hemoglobin values were 32.2% and 37.5% of original levels, respectively.

In weanling rats, however, the erythrocyte and hemoglobin concentrations rose considerably by the third day after irradiation, 41% and 42%, respectively, above original levels. This indicates hemoconcentration.

Circulating Blood Cells: 2. Leucocytes. From Chart 2 *C* one can see that the total leucocyte count dropped precipitously, reaching levels at 24 hours after irradiation which were 21.6 and 16.5% of original levels for adult and weanling rats, respectively. On the third day the leucocyte counts were reduced to 3.5% of original values for both the adult and weanling rats. In view of the hemoconcentration indicated by the 41% rise in erythrocyte concentration in weanling rats, the quantities of white cells were probably reduced even more greatly in terms of absolute numbers of circulating leucocytes or in terms of a ratio of leucocytes to erythrocytes.

From the values in Table 3, one can see that, although neutrophiles were markedly reduced, the lymphocyte reduction accounted to a greater extent for the decline

TABLE 3.—*Average Hematologic Values for Adult and Weanling Rats Receiving a Single Dose (800 r) of X-Rays**

Days Post Irradiation	No. of Rats (N)	Red Blood Count, Millions per Cu. Mm.		Hemoglobin, Gm./100 Cc.		White Blood Count, Thousands per Cu. Mm.		Neutrophiles, %		Lymphocytes, %		Neutrophiles, Thousands per Cu. Mm.		Lymphocytes, Thousands per Cu. Mm.	
		A	W	A	W	A	W	A	W	A	W	A	W	A	W
0	14 A 12 W	9.3	6.1	14.4	9.5	18,318	10,104	35.9	24.6	58.5	75.0	6,576	2,485	10,716	7,578
1	14 A 12 W	8.0	6.1	12.8	9.7	3,953	1,067†	88.1	93.8	10.1	6.1	3,482	1,563	399	101
2	12 A 10 W	8.1	6.8	12.8	10.7	2,671	471	89.5	X	9.3	X	2,390	X	248	X
3	10 A 8 W	7.9	8.6	12.4	13.5	655	358†	X	X	X	X	X	X	X	X
4	8 A	7.8	—	12.3	—	299	—	X	—	X	—	X	—	X	—
6	6 A	7.5	—	11.7	—	436	—	X	—	X	—	X	—	X	—
8	4 A	6.3	—	9.0	—	483	—	X	—	X	—	X	—	X	—
10	2 A	6.3	—	9.0	—	2,700	—	—	—	—	—	—	—	—	—

* Key to symbols: A, adult rats; W, weanling rats; X, too few cells for significant determination; —, too few or no animals surviving.
† One less than N.

of the leucocyte count. By the 24th hour, the lymphocyte counts had dropped to 3.7 and 1.3% of the preirradiation values for adults and weanlings, respectively. At this interval the neutrophile counts were reduced to 52.9 and 62.9% for adults and weanlings, respectively. By the second day the adult neutrophile and lymphocyte counts had declined to 36.3 and 2.3%, respectively. There were too few leucocytes in weanling blood at this interval and thereafter and in adult blood after the second day to provide reliable differential counts. However, it is evident from the values obtained in the first two days that a marked reversal of the lymphocyte-neutrophile ratio occurred.

The adult leucocyte count was lowest on the 4th day (1.6% of original value); it revealed only slight recovery on the 6th and 8th days, and in the two remaining rats on the 10th day it reached an average level of 14.7% of normal.

COMMENT

It is clear from the clinical observations presented that a single total-body dose of 800 r of unfiltered x-rays, under the conditions of this experiment, produced a markedly greater degree of acute illness in weanling rats than in adult rats.

In comparison of the two groups, the weanling rats ingested little food or water, became greatly lethargic, exhibited more severe diarrhea, lost weight precipitously, became prostrate soon after irradiation, and died in relatively large numbers between the third and fourth days after irradiation. In the adult group, food intake was reduced considerably for only two days, but not completely, and water intake was not reduced. Adult rats were sometimes moderately lethargic but never prostrate. The diarrhea observed in the first few days in adult rats was only moderate and discontinuous. None of the adult rats died spontaneously. All were sacrificed at intervals over a period of 10 days after irradiation.

The reductions of erythrocyte and hemoglobin concentrations in the blood of irradiated adult rats may be related to destruction and cessation of production or delivery to the blood of erythrocytes, since the bone marrow became markedly hypoplastic, or possibly it was related to hemodilution, or both. The rise of erythrocyte and hemoglobin concentrations in the blood of irradiated weanling rats, however, in view of the even more rapid and profound destruction and depletion of bone marrow, seems best explained on the basis of hemoconcentration. The clinical observations of markedly reduced water intake and severe diarrhea in weanling rats suggest that dehydration of the rats was related causally to the hemoconcentration. The hemoconcentration and the behavior of the weanling rats strongly suggest that these rats were in the shock state.

The first question which arises embodies the principal purpose of the experiment and asks whether the above differences in clinical behavior, physiologic response, and mortality between adult and weanling rats must be explained on the basis of some difference in physiologic response or reaction to quantitatively similar radiation damage, or whether it is possible to explain the differences, at least partly, on the basis of greater and more rapid damage of some or all of the tissues of the weanling rat than of adult rats after the same dose of x-rays.

It is clear from the results of the histopathologic examination of the radiosensitive tissues that damage of the intestines, spleen, lymph nodes, bone marrow, and testes was considerably greater in weanling rats than in adult rats at comparable intervals after a single total-body dose of 800 r of unfiltered x-rays. Most of the weanling rats did not survive until the fourth day, i. e., they did not survive the period (third day) of maximum intestinal damage and almost extreme depletion of bone marrow and lymphatic tissue. In adult rats, intestinal and hematopoietic damage did not progress as rapidly or become as marked. The adults recovered from the more moderate intestinal damage before the bone marrow reached a state of marked hypoplasia and while the only moderately affected lymphatic tissues were maintaining lowered cell populations, so that all of these insults were not maximum at the same time. Subsequently, hematopoietic tissues began to regenerate in the adult rats. The appearance in adult rats of hemorrhages in lymph nodes after the fourth day and the progressively declining erythrocyte and hemoglobin concentrations in the blood foreshadowed, however, the development of a mechanism of death different from that apparent in the weanling rats.

Prosser,[2] in a paper on clinical patterns leading to death after irradiation, indicates that in the shock-like death seen within 48 hours after x-irradiation in rabbits,

2. Prosser, C. L., and others: Plutonium Project: The Clinical Sequence of Physiological Effects of Ionizing Radiation in Animals, Radiology 49:299-312 (Sept.) 1947.

prostration, vomiting, and anorexia are general symptoms and that in early deaths after high doses in dogs and rats (four to six days) there is evidence for dehydration, hemoconcentration, much gastrointestinal damage, and leucopenia. The author states further that in acute deaths caused by irradiation, which comprises most of the deaths from all types of ionizing radiations and occur 9 to 21 days after treatment, there is severe leucopenia, tissue breakdown, a tendency to hemorrhage, anemia, and toxemia, among other changes.

The results suggest strongly that the differences in clinical behavior, physiologic response, and mortality between adult and weanling rats receiving a single total-body dose of 800 r of x-rays under identical conditions may be explained on the basis of greater histopathologic damage of tissues following irradiation, without the necessity of postulating a difference in physiologic response to a quantitatively similar radiation damage. The smaller amount of tissue destruction in adult rats was apparently not sufficient to cause the marked diarrhea, prostration, dehydration, hemoconcentration, or the shock-like state in which the weanling rats appeared to be involved.

The second question to arise asks whether or not differences in the internal dose can account for the difference in degree of cellular damage between adult and weanling rats. On the basis of experience in histopathologic investigations of rats of various ages that are given a wide variety of x-ray doses, it is the author's impression that a difference of 150 r in abdominal or internal dose in the dose range from 600 r to 800 r is sufficient to account for a considerable part but not all of the difference in degree of histopathologic damage observed. In general, the radiosensitivity of the tissues studied appeared to be considerably greater in the weanling rats than in the adults, and this appeared to contribute partly to the greater damage in weanling rats. The tissue destruction seen in adult rats in this experiment resembled more closely that observed in adult rats subjected to a total-body dose of 500 r of 250 kv. x-rays filtered by an aluminum parabolic filter and 0.5 mm. Cu (half-value layer of generator, 2.1 mm. Cu). The tissue destruction seen in the weanling rats in this experiment, however, resembled more closely that observed in adult rats subjected to a total-body dose of 1000 r or greater of such-filtered x-rays.

SUMMARY

Histopathologic, hematologic, and clinical data on adult and weanling rats subjected to a single total-body dose of 800 r of x-rays (250 kv., unfiltered) are presented. Measurements of dose made in air and intra-abdominally by means of Victoreen r-meters are compared.

Weanling rats exhibited a markedly greater degree of acute illness than did adult rats, ingesting little food or water and showing severe diarrhea, precipitous weight loss, prostration, and high mortality between the third and fourth days. These animals revealed hemoconcentration associated with probable dehydration and presented clinically a shock-like picture.

An explanation for the differences in clinical behavior, physiologic reaction, and mortality between adult and weanling rats was evident in the form of sufficiently greater histopathologic damage of many of the tissues examined, the tissues including intestines, spleen, lymph nodes, bone marrow, testes, and peripheral blood. Pathologic changes in various tissues of weanling rats became maximal at nearly

the same times after irradiation, whereas in adult rats maximum changes in various tissues were separated in time, reducing the stress on the animal at any particular time.

It appears to be unnecessary to postulate a difference in physiologic response to radiation to explain clinical and mortality differences between adult and weanling rats.

The greater cellular damage in weanling rats appears to be related only in part to a greater internal dose measurement and partly to greater radiosensitivity of many of the tissues examined.

During recovery of adult rats from initial tissue damage, the appearance of hemorrhages in lymph nodes and the progressively declining erythrocyte and hemoglobin concentrations in the blood suggested the possibility of later death by a mechanism different from that apparent in weanling rats.

Miss Mary Elizabeth Hudson, Miss A. Ethel Dillon, and Mrs. Florence Van Slyke gave their technical assistance in the preparation of this paper.

SPONTANEOUS CRYPTOCOCCOSIS OF MARMOSET MONKEYS IN PANAMA

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AND
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CRYPTOCOCCOSIS, or torulosis, is an infectious disease caused by a species of yeast-like fungus, Cryptococcus neoformans.[1] This yeast reproduces by budding only, both in tissues and in culture, without endospore or mycelium formation. In human disease these organisms tend to localize in the central nervous system where they produce a chronic cystic meningoencephalitis. However, systemic involvement is not uncommon.[2] Many patients have an acute fulminating course and die within six months of the onset of symptoms. If this initial stage is survived, there follows a chronic phase with a duration of two to four years or longer.[2] The mode of infection is not definitely known, but the respiratory tract is probably the primary portal of entry. Once established, hematogenous dissemination occurs.[3]

The histopathology of the disease in the central nervous system is pathognomonic. There is patchy fibrosis of the meninges, with an irregular infiltration of giant cells, lymphocytes, and plasma cells. Neutrophilic leucocytes are absent. The yeast cells are numerous and not only occur in the cytoplasm of the mononuclear and giant cells but also lie free in tissue spaces. Similar reactions occur in choroid plexus. The yeast cells enter the cerebrospinal circulation. The lesions in the brain substance itself are singularly lacking in tissue reaction. Here the organisms tend to lie within cystic spaces embedded in a gelatinous matrix. When other tissues are invaded, either the organisms may precipitate a response similar to that found in the meninges or there may be practically no reaction, as in the brain. Granulomatous lesions, similar to those of tuberculosis, have been described.

Yeast cells from human infections have proved pathogenic for white mice and rats and to a less extent for rabbits.[4] Dogs and guinea pigs are relatively resistant

From the Section of Pathologic Anatomy, Board of Health Laboratory, Gorgas Hospital.

1. Manual of Clinical Mycology: Prepared for National Research Council, Division of Medical Sciences, by N. F. Conant; D. S. Martin; D. T. Smith; R. D. Baker, and J. L. Callaway, Philadelphia, W. B. Saunders Company, 1944, Chap. 7.

2. Voyles, G. Q., and Beck, E. M.: Systemic Infection Due to Torula Histolytica (Cryptococcus Hominis): I. Report of 4 Cases and Review of the Literature, Arch. Int. Med. **77**:504, 1946.

3. Wade, L. J., and Stevenson, L. D.: Torula Infection, Yale J. Biol. & Med. **13**:467, 1941.

4. Reilly, E. B., and Artman, E. L.: Cryptococcosis: Report of a Case and Experimental Studies, Arch. Int. Med. **81**:1, 1948.

to the disease,[5] and it has not been possible to infect house cats, even with intrathecal inoculation.[6] Cutaneous cryptococcosis was produced in a Macacus rhesus monkey by injecting the organisms into the skin. The disease remained localized, however, despite simultaneous intracardiac and intraperitoneal introduction of the organisms.[7] Kessel and Holtzwart have produced meningoencephalitis in Macacus rhesus by intracardiac injections of the yeasts from human infections.[8]

The available literature records spontaneous infections with Cryptococcus in but two species of lower animals. Frothingham isolated these yeasts from tumor-like granulomatous lesions in the lungs of a horse.[9] Generalized cryptococcosis was described in a cheetah, Cynaelurus jubatus, which died at the Philadelphia Zoological Gardens.[6] At autopsy the lesions resembled a widespread neoplasm. Later, a second cheetah succumbed there to the same disease.[10] We are not aware of any reported cases of spontaneously acquired infections in monkeys. The following account of spontaneous cryptococcosis of the marmoset monkey (Leontocebus geoffroyi) in Panama adds a third lower animal host species to those previously encountered.

REPORT OF CASES

History.—The cases reported here were found in a male and female marmoset monkey purchased in Panama about three months prior to their death. The monkeys resisted handling and remained in a cage most of the time following acquisition. No symptoms were noted before death. The male was found dead in the cage on Oct. 18, 1952, and the female on the following day. The animals were refrigerated and brought to the Board of Health Laboratory for autopsy on Oct. 20.

Necropsy.—Both animals were sexually immature and had attained about three-quarters of the adult size at the time of death. The female (#6584) measured 18 in. (45.72 cm.) in total length; the male (#6585) was 18.3 in. (46.48 cm.) long. Both animals were emaciated. No skin lesions were present; no ectoparasites were found. The anatomical findings will be summarized jointly since they were similar for the two monkeys.

The lungs were congested, with a rubbery consolidation throughout, and presented a deep purplish black color. All heart chambers were markedly dilated and filled with coagulated blood. The spleens were enlarged, were ovoid in shape, and had a soft jelly-like consistency. The other viscera were not remarkable, except for the mesenteric lymph nodes. These were greatly enlarged, were pale gray, and ranged from 3 to 15 mm. in diameter. On sectioning of the nodes after fixation, a clear semiliquid gelatinous material oozed from the cut surfaces. The normal nodal architecture was obliterated.

At autopsy the condition was diagnosed grossly as a severe generalized pneumonitis, a common cause of death in captive monkeys. Consequently, no examination of the central nervous system was conducted.

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5. Beck, E. M., and Voyles, G. Q.: Systemic Infection Due to Torula Histolytica (Cryptococcus Hominis): II. Effect of Chemotherapeutic Agents in Experimentally Produced Infections, Arch. Int. Med. **77**:516, 1946.

6. Weidman, F. D., and Ratcliffe, H. L.: Extensive Generalized Torulosis in a Chetah or Hunting Leopard (Cynaelurus Jubatus), Arch. Path. **18**:362, 1934.

7. Weidman, F. D.: Cutaneous Torulosis: Identification of Yeast Cells in General in Histologic Sections, South. M. J. **26**:851, 1933.

8. Kessel, J. F., and Holtzwart, F.: Experimental Studies with Torula from a Knee Infection in Man, Am. J. Trop. Med. **15**:467, 1935.

9. Frothingham, L.: A Tumor-Like Lesion in the Lung of a Horse Caused by a Blastomyces (Torula), J. M. Res. **9**:31, 1902.

10. Weidman, F. D.: Personal communication to the authors.

Microscopic Examination.—Lung: The majority of the alveoli were filled with an exudate composed of plasma cells and lymphocytes, while some were filled with extravasated blood. Numerous macrophages and a few multinucleated giant cells were also found. Throughout the areas of inflammation there were doubly refringent bodies surrounded by a relatively thick capsule. These ranged from 3 to 8 μ in diameter, and the capsules were from 1.2 to 2.5 μ in thickness. The capsules sometimes stained with eosin, but the cells rarely took the stain. Many budding cells were found. The organisms were usually solitary, but occasional clumps of two or three were seen.

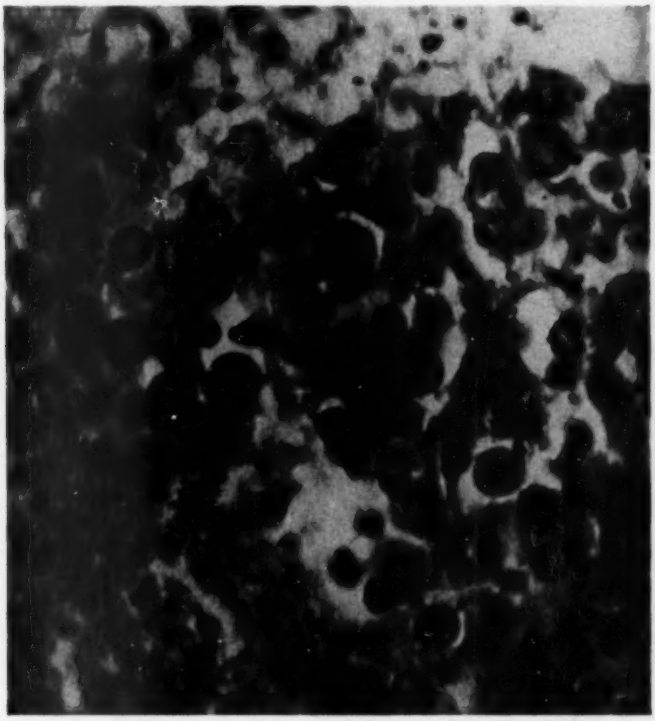

Fig. 1.—Cryptococcus organisms in a section of lung from a marmoset monkey (#6585); hematoxylin and eosin.

Spleen: The sinusoidal areas contained a network of fine eosinophilic fibrils. The interstices contained yeast forms similar to those found in the lungs, except that very few showed capsular staining. There were large, apparently empty spaces surrounding the parasites. Inflammation was practically absent. Occasionally the yeast cells could be found in lacunae within the germinal centers. The splenic capsule and trabeculae were not invaded.

Small Intestine: There was patchy denudation of the mucosa. These denuded areas contained networks of fibrils and scattered yeast parasites in lesions similar to those seen in the spleen. There was no significant inflammation.

Lymph Nodes: The nodal architecture was more or less completely replaced with zones similar to those seen in the spleen and small intestine. Unstained encapsulated bodies were common. Inflammatory reaction was lacking within the nodes, but foci of mononuclear cells occurred in the perilymphatic adipose tissue.

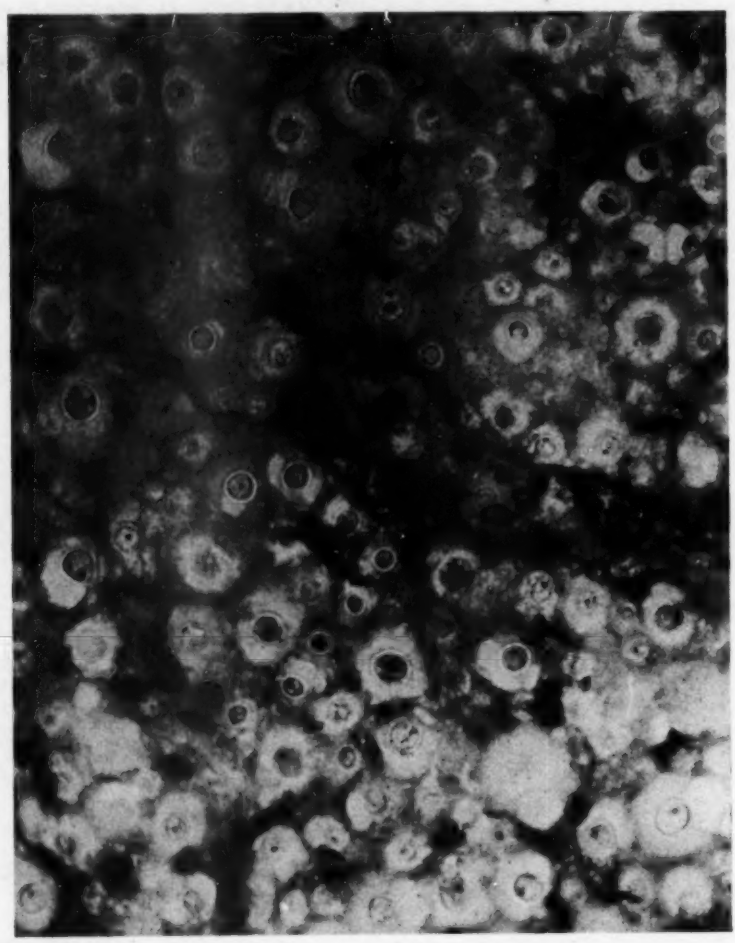

Fig. 2.—The lesions of cryptococcosis in a section of spleen from Leontocebus geoffroyi (#6584); hematoxylin and eosin.

Other Tissues: The lobular architecture of the liver was preserved, but numerous minute foci of mononuclear cells were present. Yeast cells were associated with only a few of these foci. Similar areas were seen in the kidneys, but no organisms were found in the exudate. Isolated yeast cells were located in the glomerular capillaries, as well as vessels of the liver, myocardium, parotid glands, adrenal glands, and the pancreas.

The Organism: On morphologic and histopathologic evidence, these organisms are believed to be yeasts closely allied to Cryptococcus neoformans, if not the same species. Attempts to culture the yeasts from the spleen and lungs were unsuccessful, as the cultures were overgrown by Candida albicans. Dr. F. D. Weidman, who kindly examined the sections, agreed that the organisms present were Cryptococcus.[10] The cells and capsules stain a deep lavender color with the Hotchkiss-McManus stain.

COMMENT

The lung lesions in the marmoset monkeys are similar to those described from human cases of generalized cryptococcosis. They differ only in the intensity and wide dissemination of the lesions, which suggests a more explosive course of the disease in the marmoset. The lesions were unlike those described from the horse and cheetah which had the gross appearance of being neoplastic. Experimentally infected Macacus rhesus monkeys have failed to demonstrate lesions of the lungs, liver, and spleen, while other organs yielded characteristic lesions. We have no explanation for these differences in the manifestations of the disease except the obvious one of species variation.

No cases of cryptococcosis have been noted among the more than 19,000 autopsies on humans done at the Board of Health Laboratory since 1904. Furthermore, the disease has not been diagnosed in Gorgas Hospital patients, or, to the best of our knowledge, has it been found at Santo Tomas Hospital in Panama. Complete autopsies of more than 65 wild monkeys of various species, in the course of yellow fever studies, likewise failed to disclose lesions of cryptococcosis. Therefore, we believe that these two cases in the marmoset monkeys represent the first infections to be recognized on the Isthmus.

SUMMARY

Two cases of spontaneous infection with Cryptococcus are described from the marmoset monkey, Leontocebus geoffroyi, of Panama.

This is the third species of lower animal to be reported as having acquired the infection spontaneously.

These are the first cases of cryptococcosis to be described from the Isthmus of Panama.

Photographs were taken by H. A. Dunn.

MYOCARDITIS IN POLIOMYELITIS

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THE FINDING of acute myocarditis in patients dying of poliomyelitis has suggested the possibility that cardiac failure may be a principal or contributing cause of death in this disease. Although earlier isolated reports of myocardial alterations are recorded,[1] it remained for Saphir and Wile [2] to emphasize the frequency and potential importance of these lesions. Subsequent reports [3] have verified these observations (Table 1).

It is not definitely known that the myocardial lesions are due to a direct action of the poliomyelitis virus, but the fact that Jungeblut and Edwards [3h] have isolated the virus from the hearts of three patients who died during the epidemic of 1949 suggests this. Furthermore, Jungeblut and Steenberg [4] have demonstrated experimentally that both the mouse-adapted Columbia SK and the encephalomyocarditis viruses may show viscerotropic, as well as neurotropic, properties. In addition, Ludden and Edwards [3e] have noticed in their studies of poliomyelitis cases in which autopsies were done that those with myocarditis occurred in chronologic sequence,

From the Department of Pathology, Northwestern University Medical School, and the Department of Pathology, Evanston Hospital, Evanston, Ill.

1. Robertson, H. E., and Chesley, A. J.: Pathology and Bacteriology of Acute Anterior Poliomyelitis, Arch. Int. Med. **5**:233-269, 1910. Abramson, H. L.: Pathologic Report on 43 Cases of Acute Poliomyelitis, ibid. **22**:312-330, 1918. Landon, J. F., and Smith, L. W.: Poliomyelitis: A Handbook for Physicians and Medical Students Based on a Study of the 1931 Epidemic in New York City, New York, The Macmillan Company, 1934. Dublin, W. B., and Larson, C. P.: Pathologic Findings in Poliomyelitis, Am. J. Clin. Path. **13**:15-17, 1943. Cowie, D. M.; Parsons, J. P., and Lowenberg, K.: Clinico-Pathologic Observations on Infantile Paralysis: Report of 125 Acute Cases with Special Reference to the Therapeutic Use of Convalescent and Adult Blood Transfusions; The Possible Relation of Blood Group to Severity of the Disease, Ann. Int. Med. **8**:521-551, 1934.

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3. (*a*) Peale, A. R., and Lucchesi, P. F.: Cardiac Muscle in Poliomyelitis, Am. J. Dis. Child. **65**:733-738, 1943. (*b*) Saphir, O.: Visceral Lesions in Poliomyelitis, Am. J. Path. **21**:99-109, 1945. (*c*) Gefter, W. I., and others: The Heart in Acute Anterior Poliomyelitis, Am. Heart J. **33**:228-239, 1947. (*d*) Dolgopol, V. B., and Cragan, M. D.: Myocardial Changes in Poliomyelitis, Arch. Path. **46**:202-211, 1948. (*e*) Ludden, T. E., and Edwards, J. E.: Carditis in Poliomyelitis: An Anatomic Study of 35 Cases and Review of the Literature, Am. J. Path. **25**:357-381, 1949. (*f*) Spain, D. M.; Bradess, V. A., and Parsonnet, V.: Myocarditis in Poliomyelitis, Am. Heart J. **40**:336-344, 1950. (*g*) Bradford, H. A., and Anderson, L. L.: Electrocardiographic Observations During Poliomyelitis Epidemic, Ann. Int. Med. **32**:270-278, 1950. (*h*) Jungeblut, C. W., and Edwards, J. E.: Isolation of Poliomyelitis Virus from the Heart in Fatal Cases, Am. J. Clin. Path. **21**:601-623, 1951.

4. Jungeblut, C. W., and Steenberg, E.: Neurotropic and Viscerotropic Strains of Columbia SK and Encephalomyocarditis Virus, Arch. Path. **49**:574-581, 1950.

and therefore they suggested that the virus may have cardiotropic properties in some epidemics. Thus it seemed desirable to attempt to obtain further evidence either for or against the supposition that in certain epidemics there may be viscerotropic strains of poliomyelitis virus that cause myocarditis. Accordingly, the pathological material of the Evanston Hospital, where a large number of patients with poliomyelitis are treated each year, was reviewed from this point of view. In addition, certain features of the myocardial lesions that are of general interest will be discussed.

MATERIAL AND METHODS

The material for this report consists of 47 fatal cases in which autopsies were done from 1942 to 1951, inclusive. In all of them, typical spinal and bulbar lesions of acute anterior poliomyelitis were present. Sections of the myocardium were fixed in Zenker's or alcohol-formol solutions, embedded in paraffin, and stained with hematoxylin and eosin. Since the material was from routine autopsies, the same number of sections was not available in all cases. In 12 cases, there was only one section of myocardium; in 15 cases, two sections; in 14 cases, three sections; in 4 cases, four sections, and in 2 cases, five sections. The severity of the myocarditis was graded on an increasing scale from 1 to 3 +.

TABLE 1.—*Summary of Reported Series of Poliomyelitis Myocarditis*

Author	Yr. Published	No. of Cases	Myocarditis	Cases with Myocarditis, %
Saphir and Wile [3a]	1942	7	6	86
Peale and Lucchesi [3a]	1943	9	7	78
Saphir [3b]	1945	17	10	60
Gefter and others [3c]	1947	6	2	33
Dolgopol and Cragan [3d]	1948	92	16	17
Ludden and Edwards [3e]	1949	35	14	40
Spain, Bradess, and Parsonnet [3f]	1950	14	12	86
Bradford and Anderson [3g]	1950	24	3	12.5
Jungeblut and Edwards [3h]	1951	5	2	40
Teloh	1952	47	26	55
Summary of reported cases		206	98	42.4

RESULTS

Sex and Age Incidence.—The 47 patients included 32 males and 15 females, corresponding to the incidence noted by Ludden and Edwards.[3e] There was no significant difference in the incidence or in the severity of the myocardial lesions. The range of ages varied from 10 months to 50 years, with a concentration of cases in the younger age groups. No significant variation in the incidence of myocarditis was noted in any age group.

Annual Incidence.—Tabulation of the data by years showed a greater incidence of myocarditis during the autumn of 1949, as compared with a fairly constant rate of occurrence during preceding and subsequent years. It is also notable that during 1949 the lesions were severer than in the other years. These findings suggest the idea that there may be a cardiotropic strain of poliomyelitis, but, before one accepts such a conclusion, more data will have to be collected.

Cardiac Enlargement.—A slight but significant increase in heart weight has been reported in poliomyelitis and attributed to edema of the myocardium.[3e] In this

series, 13 of the 47 hearts exceeded by at least 25% the expected normal weight,[5] and in none of them was there any other disease which might explain the increase. Only four (31%) of the hypertrophied hearts had myocarditis, and thus the enlargement in some of the cases could not be explained on the basis of inflammation. On the other hand, of the 34 hearts of normal weight, 22 (65%) had myocarditis. Thus some other factor besides inflammation must have caused the cardiac enlargement, but the theory that it was edema could not be proved.

Dilatation of the heart was present in 17 cases but was usually not marked. There was no demonstrable association between the dilatation and the occurrence or severity of myocarditis.

Microscopic Features of the Myocarditis.—The myocarditis was exudative and limited to the interstitial tissues. The myocardial fibers were regularly spared, and only rarely was there necrosis. Except for cloudy swelling in a few cases, degenerative changes were uncommon. In mild involvement, the exudate was commonly perivascular and composed of small numbers of lymphocytes, plasma cells, macrophages, and polymorphonuclear leucocytes in varying proportions. Proliferation of fixed tissue cells and accumulations of Anitschkow myocytes were frequently observed. The lesions were usually focal and separated by large zones of normal-appearing myocardium. In severe cases, the cellular exudate was more abundant, with polymorphonuclear leucocytes predominating. Edema also was marked. Instead of being predominantly focal, the involvement was often diffuse, and the exudate extended between the fibers and separated them, but the apparent histological integrity of the individual fibers was a striking feature.

TABLE 2.—*Yearly Incidence of Myocarditis*

Occurrence, Yr.	No. of Cases	Degree of Myocarditis				Cases with Myocarditis, %
		0	1+	2+	3+	
1942	1	1	..	..	..	0
1943	3	2	1	..	..	33
1944	4	2	2	..	..	50
1945	5	3	..	2	..	40
1946	11	6	3	1	1	45
1947	..	..	..	..	..	..
1948	..	..	..	..	..	..
1949	6	..	3	1	2	100
1950	5	3	2	..	..	40
1951	12	4	7	1	..	66

COMMENT

The results of this study show that the incidence of myocarditis in patients dying of poliomyelitis in the Evanston Hospital during the epidemic of 1949 was 100%, in contrast to the much lower incidence in the years 1942 to 1946 and in 1950 and 1951, when it varied from 33 to 66%. Thus, this study affords additional evidence that viscerotropic strains of the poliomyelitis virus may exist and that these may specifically attack the myocardium and be more prevalent or virulent in some

5. Saphir, O.: Autopsy Diagnosis and Technic, Ed. 2, New York, Paul B. Hoeber, Inc., 1946.

epidemics than in others.[6] Final proof of this idea and elucidation of the mechanism of formation of such strains and their possible mode of action must, of course, await further study.

Although the frequent occurrence of myocarditis in patients dying of poliomyelitis seems well established; its significance is not fully understood. In many of the cases examined in this study, as well as in those reported in the literature, the inflammation is minimal and focal in distribution, and this fact, of course, raises a question of its functional significance. It has not been possible in this investigation to establish a good correlation between cardiac dilatation and hypertrophy and the occurrence of myocarditis. These findings suggest that the myocarditis may be of only incidental importance.

The mechanism of the development of the myocarditis remains unknown.

SUMMARY AND CONCLUSIONS

A review of 47 fatal cases of acute anterior poliomyelitis in which autopsies were done at the Evanston Hospital during the years 1942 to 1951, inclusive, showed that in 26 (55.3%) there was acute myocarditis, as determined by microscopic examination of routine histologic sections.

During the epidemic of 1949, the incidence of myocarditis was much greater than in any other year (100%), and it was also of greater severity. This is additional evidence to that collected by other authors, that during certain epidemics, there are viscerotropic strains of the virus which may injure the myocardium.

The occurrence of acute myocarditis could not be correlated with the sex or age of the patient or with the occurrence of cardiac dilatation or hypertrophy.

6. Ludden and Edwards.[3e] Jungeblut and Steenberg.[4]

EXPERIMENTAL ABERRANT MEDIASTINAL GOITERS (THYMIC) IN THE RAT

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THE INFREQUENT occurrence of spontaneous neoplasms in the thymus gland of Wistar rats is well recognized (Ratcliffe [1]). The purpose of the present report, therefore, is to call attention to the development of thyroid-like nodules (tumors) associated with the thymus gland in Wistar stock colony rats subjected to prolonged influence of the goitrogen, thiourea. These may be of considerable interest, for neoplasms of thymic origin are uncommon (Struthers,[2] Seybold and others [3]), and metastatic tumors in the thymus, at least in the human being, are rare (Karsner [4]). The implication that accessory thyroid tissue may be induced to form in the thymus takes on added significance in view of the therapeutic use of drugs with antithyroid action in the control of human thyrotoxicosis.

A review of the more recent literature indicates that only occasional isolated examples of human thymic neoplasia have been recorded. Some of these tumors, especially the benign tumors, are associated with myasthenia gravis (Blalock and others [5]). On the other hand, malignant neoplasms of the thymus are rare (Rider and McDonald [6]). The pleomorphism of human thymic tumors, however, is responsible for considerable difficulty in their classification; yet they appear to be largely epithelial in origin. Although McDonald,[7] Slesinger,[8] and Norris [9] have described

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1. Ratcliffe, H. L.: Spontaneous Tumors in 2 Colonies of Rats of the Wistar Institute of Anatomy and Biology, Am. J. Path. **16**:237-254, 1940.

2. Struthers, R. R.: Teratoma of the Thymus Gland: Report of a Case, Canad. M. A. J. **26**:68-70, 1932.

3. Seybold, W. D.; McDonald, J. R.; Clagett, O. T., and Good, C. A.: Tumors of the Thymus, J. Thoracic Surg. **20**:195-215, 1950.

4. Karsner, H. T.: Human Pathology, Ed. 6, Philadelphia, J. B. Lippincott Company, 1942.

5. Blalock, A.; Mason, M. F.; Morgan, H. J., and Riven, S. S.: Myasthenia Gravis and Tumors of the Thymic Region: Report of a Case in Which the Tumor Was Removed, Ann. Surg. **110**:544-561, 1939.

6. Rider, J. A., and McDonald, R.: Myasthenia Gravis in a Case of Malignant Thymoma Resistant to Neostigmine Therapy, Am. J. M. Sc. **219**:71-75, 1950.

7. McDonald, S., Jr.: A Case of Reticulum Cell Carcinoma of the Thymus, J. Path. & Bact. **35**:1-9, 1932.

8. Slesinger, H. A.: Primary Carcinoma of the Thymus Gland: Case Report, J. Lab. & Clin. Med. **22**:151-155, 1936.

9. Norris, E. H.: A Thymoma (Adenoma of the Thymus) from an Unusual Case of Myasthenia Gravis, with Observations on the General Pathology, Am. J. Cancer **30**:308-317, 1937.

an alveolar-like arrangement of the reticulum cells in their reports of thymic neoplasia, few thymic tumors possess distinct adenomatous character. According to Seybold and others,[3] "all thymoma contain the thymic lymphocyte or thymocyte and thymic epithelial cells in varying proportions."

It is generally agreed that most human mediastinal thyroid tumors are outgrowths from the isthmus or lower pole of the parent thyroid gland (Lahey[10]). These migrating adenomatous (nodular) growths frequently have a vascular pedicle (usually a branch of the inferior thyroid artery) which maintains a connection with the main body of the thyroid gland. These goiters, which may be partially, as well as completely, intrathoracic, are not uncommon (McGavack[11]).

In recognizing another group of mediastinal thyroids, McGavack states that embryonic follicular rests of thyroid tissue, located near the arch of the aorta, have no clinical significance. While aberrant thyroid tissue is not infrequent in the neck, aberrant intrathoracic goiters are considered very rare (Rives[12]). All but 7 of 19 human mediastinal aberrant goiters reported in the literature were found in the anterior mediastinum (Mora and others,[13] Rives,[12] Bradford and others,[14] Nègre and Balmès[15]). The location of 13 of these made it unlikely that they arose from the main body of the thyroid gland during extrauterine life. In some, a blood supply from an intrathoracic vessel, rather than a branch of a thyroid artery, could be identified. Five of six similar tumors located in the superior mediastinum showed no connection whatever with the neck. Rives,[12] in his review of such tumors, states that they are nodular goiters, behaving the same as adenomatous goiters elsewhere, and that the majority were accompanied by nodular goiters in the neck. This investigator points out that these intrathoracic tumors do not arise by migration from nodular adenomata in the thyroid gland, but he does not postulate their origin. He does not believe they represent metastases. Presumably, such aberrant mediastinal goiters have been assigned to embryonic rests of thyroid tissue. Moreover, Rives believed that they were probably much more frequent than commonly recognized.

In support of this concept are the numerous thyroid-like adenomata (goiters) in the thymus of rats following administration of thiourea. They were encountered during a recent study dealing with experimental neoplasia of the thyroid gland (Van Dyke[16]). Although such nodules might be explained on the basis of metastases from associated thyroid tumors, they are most readily assigned to persisting embryonic thyroid "rests" which have been augmented by the action of thiourea. Certain evidence to be presented here, however, also suggests the possibility of an origin for these accessory thyroid bodies from the epithelial component of the thymus gland.

10. Lahey, F. H.: Intrathoracic Goiters, S. Clin. North America **25**:609-618, 1945.

11. McGavack, T. H.: The Thyroid, St. Louis, C. V. Mosby Company, 1951.

12. Rives, J. D.: Mediastinal Aberrant Goiter, Ann. Surg. **126**:797-810, 1947.

13. Mora, J. M.; Isaacs, H. J.; Spencer, S. H., and Edidin, L.: Posterior Mediastinal Goiter, Surg., Gynec., & Obst. **79**:314-317, 1944.

14. Bradford, M. L.; Mahon, H. W., and Grow, J. B.: Mediastinal Cysts and Tumors, Surg., Gynec., & Obst. **87**:467-491, 1947.

15. Nègre, E., and Balmès, A.: Les goitres du médiastin postérieur, J. chir. **66**:190-200, 1950.

16. Van Dyke, J. H.: Experimental Thyroid Tumors Derived from Ultimobranchial Tissue in the Rat, Proc. Indiana Acad. Sc. (1950) **60**:313-314, 1951.

Furthermore, an examination of the literature indicates that spontaneous accessory thyroid-like masses in the thymus of the rat are apparently rare. An occasional example, however, might be expected to occur.

MATERIAL AND METHODS

A total of 31 Wistar rats ranging in age from 1 month to 6 months at the beginning of the experiment are included in the present study. Nineteen of these (12 females and 7 males) comprised the experimental group. These were maintained on a 0.2% solution of thiourea, administered as drinking water, for periods of 7 to 14 months. Twelve rats were used as untreated controls. These were divided into two groups of six, with three females and three males in each group. One control group was sacrificed at 6 months and the other at 12 months of age.

In order to demonstrate evidence of mitoses under optimum conditions, all animals were injected with a 0.01% aqueous solution of colchicine seven hours before autopsy, as prescribed by Paletta and Cowdry [17] for the mouse. The entire thymus and thyroid gland (intact with the larynx) were fixed either in Bouin's fluid, Zenker's fluid, or 10% formalin in preparation

Tumors of the Thymus and Thyroid Gland in Wistar Rats Maintained on Two-Tenths per Cent Thiourea for Seven to Fourteen Months*

	No. of Rats	Average Age, Mo.	Average Time of Experiment, Mo.	Thymic Tumors	Thyroid Tumors
Incidence based on sex					
F	12	3.5	11	5	7
M	7	1.0	12	4†	4
Incidence based on duration of treatment					
Shorter period	6	2.5	8	2	1
Extended period	13	3.0	13	7†	10
Totals	19	...	..	9 (47%)	11 (58%)

* Rats were 1 to 6 months old.
† Rats showing prevalence of thymic tumors.

for staining with hematoxylin and eosin or Mallory's connective tissue stain. Both tissues were oriented during embedding to distinguish the cranial from the caudal pole.

Complete serial sections of the thymus, cut in the frontal plane at 7 μ, were examined. Similar serial sections of the entire thyroid gland in these animals were also studied. However, since the description of these thyroid glands constitutes a large part of a separate study, they will be considered only briefly in the present account.

OBSERVATIONS AND RESULTS

The Table illustrates the total number of thymic and thyroid tumors encountered in the thiourea-treated rats. It will be noted that thymic nodules were identified in 9 (47%) of the 19 rats subjected to the goitrogen. A typical thymic goiter is shown in Figure 5. In 11 animals, or 58% of the total, neoplasms were also identified in the thyroid gland proper. Five experimental rats possessed both thymic and thyroid neoplasms.

The Table also shows the incidence of thymic neoplasia based on sex of the animals and the duration of thiourea administration. Thymic tumors appear to be somewhat more prevalent in males (57%) as compared to females (41%) and in those animals maintained on the goitrogen for extended periods (53%) as com-

17. Paletta, F. X., and Cowdry, E. V.: Influence of Colchicine During Methylcholanthrene Epidermal Carcinogenesis in Mice, Am. J. Path. **18**:291-311, 1941.

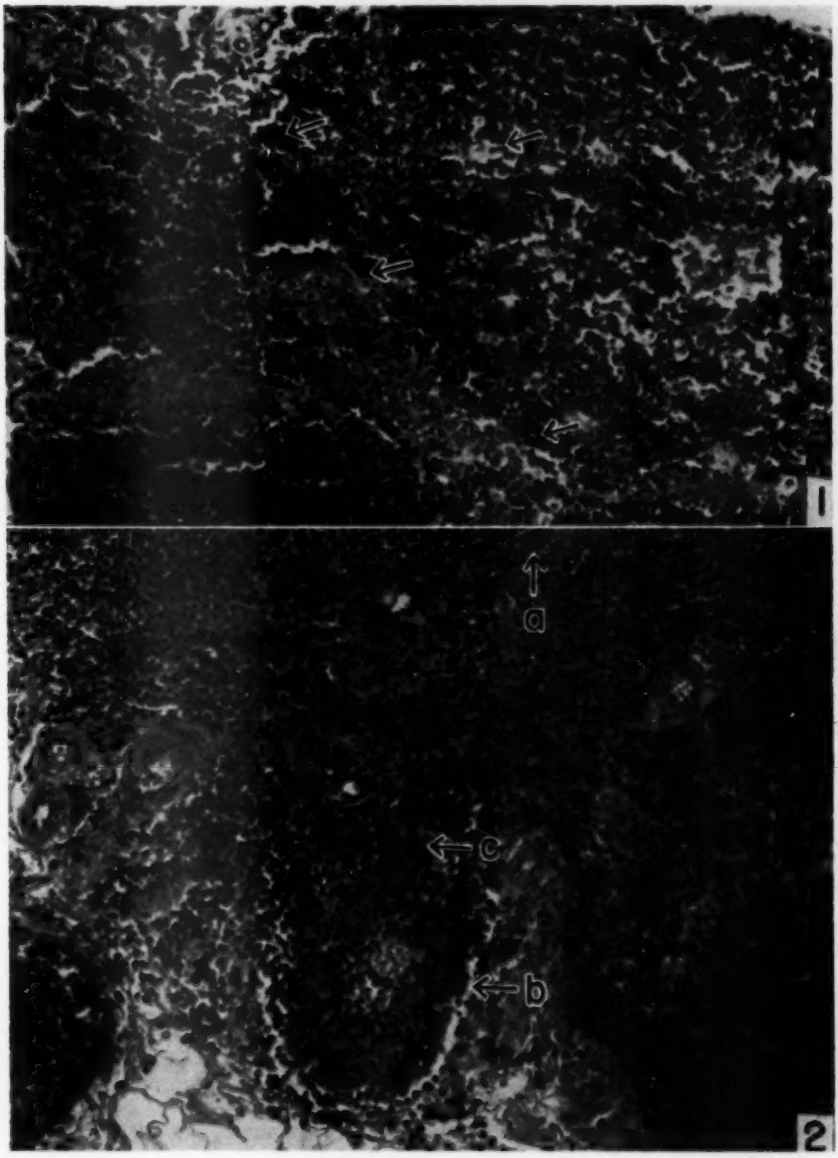


Fig. 1.—Section from atrophic thymus of female rat subjected to 0.2% thiourea for three months (age, 15 months). Note evidence of hyperplasia of reticulum cells illustrated by epithelial cell cords (arrows) in the remains of the medulla. Mitoses, while numerous, are not clear at this magnification. Hematoxylin and eosin; × 135.

Fig. 2.—Involuted thymus from female rat subjected to 0.2% thiourea for six months (age, 18 months). Reticulum cell hyperplasia also occurred in this thymus. A Hassall's corpuscle is identified (*a*). Note cluster of potential vesicles forming (*b*) and a pedicle-like extension connecting the epithelial mass to a portion of the medulla (*c*). Hematoxylin and eosin; × 160.

pared to the shorter period (33%). Most of the rats one month of age at the start of the experiment (males) were subjected to thiourea for the longest period (14 months). This probably accounts for the higher incidence of demonstrable thymic nodules in this sex and in the youngest age group.

Control Thymus Glands.—Neither the thyroid gland nor the thymus of 12 control rats showed evidence of neoplastic change. The thymus of one 6-month-old specimen contained a cyst lined by stratified squamous epithelium. This was in the medulla at the cranial end of the thymus. In three of the older rats, cysts lined by a ciliated columnar epithelium were identified also near the upper end of the thymus. These epithelial cysts were similar in structure and location to those previously described by Marine [18]; they contained a vacuolated acidophilic material. In one thymus, such a cyst lay close to an epithelioid mass with a peculiar alveolar arrangement of its cells similar to the so-called "thymic body" mentioned by Davidson.[19] Although Selye [20] and Ross and Korenchevsky [21] maintain that such epithelial cell nests and cysts are rare in the normal rat thymus, Plagge [22] found small epithelial cysts in all of six apparently normal specimens studied in serial section.

Experimental Thymus Glands.—The thymus of the thiourea-treated animals exhibited different degrees of involution. They were all characterized, however, by a decrease in the size of the gland, loss of lymphocytes, and diminished differentiation between the cortex and medulla. In general, there was an increase in the fibrous and adipose tissue content coincident with marked atrophy of the thymic parenchyma in the oldest rats. In some rats only a remnant of thymus tissue persisted.

Hassall's corpuscles, normally inconspicuous in the rat, were for the most part either few or diminutive (frequently composed of only two or three cells). However, in five rats reticulum cell hyperplasia had produced prominent cords of epithelial cells which caused the medulla to become quite conspicuous (Fig. 1, arrows). In some of these animals, Hassall's corpuscles were enlarged. The thymus of four such rats showing these "epithelioid" arrangements of hyperplastic reticulum cells possessed discrete masses of potential alveoli resembling, but apparently not identical to, the "thymic bodies" described by Davidson (Fig. 2, *b*). Evidence of hyperplasia of thymic cytoreticulum accompanied most of the thyroid-like adenomata found in thymus tissue.

Epithelial cysts, some of which showed hyperplasia, occurred in the medulla of 12 rats. Although their position was not constant, most of these cysts were located near the upper pole of the thymus gland. In five animals, such cysts were lined by stratified squamous epithelium. In seven thymus glands, they possessed largely a ciliated columnar epithelium. Occasionally these cysts were composed in part by one kind of epithelium and in part by the other. They have been interpreted, for the

18. Marine, D., in Cowdry, E. V., Editor: Special Cytology: Form and Functions of the Cell in Health and Disease, New York, Paul B. Hoeber, Inc., 1928, Vol. 1, p. 549.

19. Davidson, W. M. B.: Development of Thymic Bodies in Rats After Fat Injection, Brit. J. Exper. Path. **32**:448-451, 1951.

20. Selye, H.: Thymus and Adrenals in Response of Organism to Injuries and Intoxications, Brit. J. Exper. Path. **17**:234-248, 1936.

21. Ross, M. A., and Korenchevsky, V.: Thymus of the Rat and Sex Hormones, J. Path. & Bact. **52**:349-360, 1941.

22. Plagge, J. C.: Some Effects of Prolonged Massive Estrogen Treatment on the Rat, with Special Reference to the Thymus, Arch. Path. **42**:598-606, 1946.

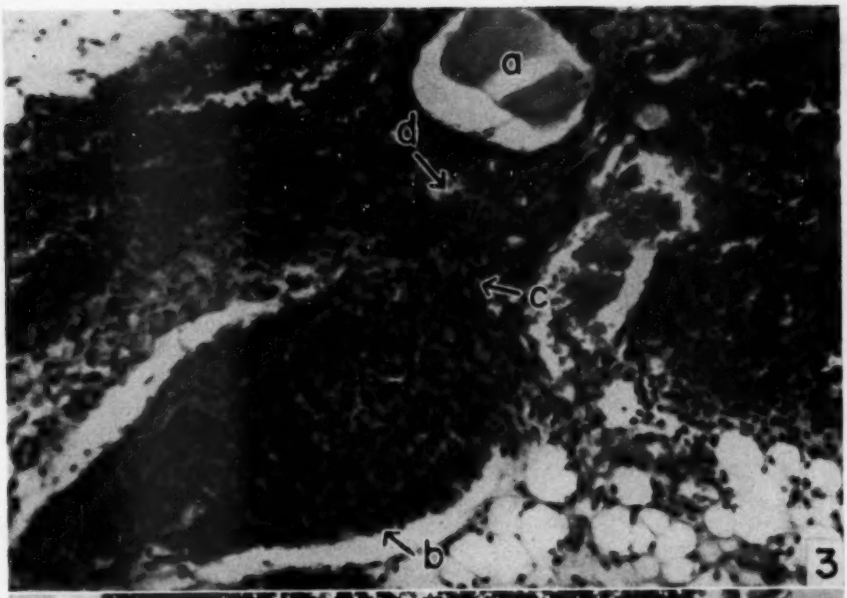


Fig. 3.—Thymus of female rat treated with thiourea for 12 months (age, 17 months). Note epithelial cyst (*a*). An incipient thymic tumor is represented (*b*), with an epithelial pedicle (*c*) contiguous with hyperplastic epithelial reticulum of the thymus (*d*). A tumor existed in the thyroid gland of this animal. Hematoxylin and eosin; × 135.

Fig. 4.—Thymus from male rat (age, 15 months) maintained for 14 months on thiourea. An epithelial cyst is indicated (*a*). A thyroid-like neoplasm (*b*) is shown to be enclosed within the connective tissue capsule of the thymus gland (*d*). Note juxtaposition of this "new-growth" to thymus tissue, the contiguity of which is shown at the level of area *c*. A tumor existed in the thyroid gland of this animal. Hematoxylin and eosin; × 80.

most part, as remnants of the "thymo-pharyngeal duct" (Speer [23]) or, possibly in the rat, the ultimobranchial body. Five thymic adenomata were found closely associated with such cysts (Figs. 3, *a*, and 4, *a*). In four animals, however, thymic nodules were found in the septa or in the capsule of the gland somewhat removed from its cranial end and unassociated with epithelial cysts.

Aberrant Mediastinal Goiters.—These accessory thyroid-like bodies varied from 1.0 to 4.0 mm. in their greatest diameter. Four of these goiters were incipient in character. Typically, they were composed of small clusters of cells with potential lumina resembling embryonic thyroid tissue (Figs. 2, *b*, and 3, *b*.). Mitoses were only occasionally encountered. Because of their small size, they gave the impression of having developed recently and only after prolonged influence of the goitrogen.

With four exceptions, these "new-growths" were contiguous with a portion of the epithelial (entodermal) component of the thymic medulla (Fig. 4, *c*). This was particularly noticeable in sections of thymus glands showing general hyperplasia of reticulum cells. In three thymus glands, an actual pedicle was identified connecting the adenomatous nodule to cords of hyperplastic entodermal cells (Fig. 3, *c*). Moreover, such pedicles apparently provided their blood supply.

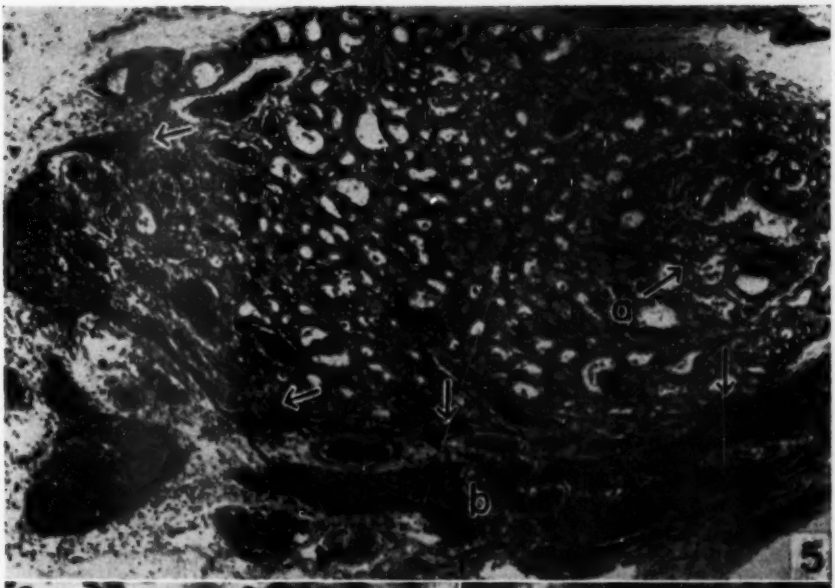
The larger, more mature tumors, of which there were five, usually resembled the thyroid gland proper; frequently they exhibited the same state of physiologic activity (Figs. 6 and 7). In two of the largest nodules, a single discrete cystadenomatous area could be identified (Fig. 5, *a*). Traces of colloid were also observed in some of the larger neoplasms. Figure 5 indicates a suggestion of four points of contiguity between thymus tissue and a mature thyroid-like nodule (arrows). Such regions of contact often were characterized by the presence of eosinophilic leucocytes. However, since there was little or no suggestion of preexisting pedicles in some relatively mature thymic tumors, these sites of interconnection between neoplasm and thymus tissue may be transient.

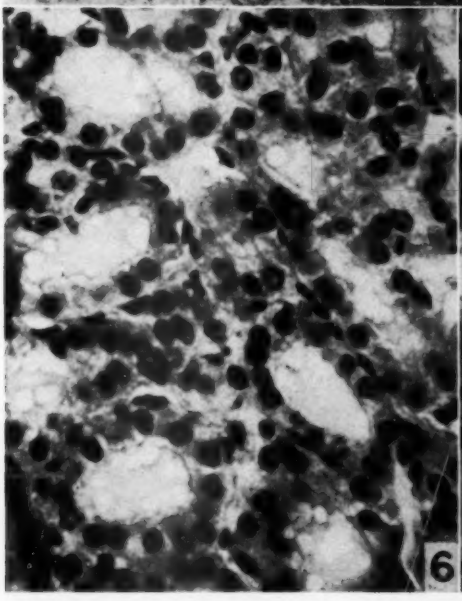
Multiple growths occurred in the thymus of two animals. In one of these, the nodules were relatively close together, suggesting a splitting of the original tumor into two or more masses or an origin essentially from the same region of the thymus.

All aberrant mediastinal thyroids were associated with the thymus gland proper. In some instances thymic lymphocytes were intermingled with the parenchyma of the epithelial "new-growth" (Fig. 4). Except in the region of contiguity with thymic epithelium, all of these nodules were encapsulated and appeared benign (Fig. 4, *d*). Mitotic figures, while relatively scarce, were more frequent in the incipient tumors. There was no real similarity between the neoplasms in the thyroid glands of these animals and those found in the thymus. Thymic adenomata, as a whole, were highly vascular and resembled goitrous accessory thyroid tissue. Because of the formation of pedicles, they appeared to arise from the epithelial component of the thymus gland.

Thyroid Adenomata.—Tumors of the thyroid gland, in five animals coincident with thymic neoplasia, were of two essential kinds. Most of these adenomata (seven) were papillary cystadenomata. These were densely staining, highly vascular neoplasms surrounded by a delicate connective tissue capsule. In other thyroid glands (four), the tumor had the character of a solid epithelioid body with small, thin, and

23. Speer, F. D.: Thymic Cysts: Report of a Thymus Presenting Cysts of 3 Types, Bull. N. Y. M. Coll. **1**:142-150, 1938.





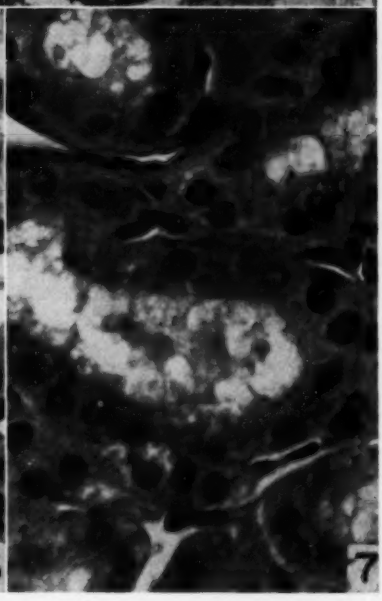


Fig. 5.—Low-power magnification of a thymic goiter in a female rat (age, 17 months) treated for 12 months with thiourea. Note papilliferous locus (*a*) and the remnants of the thymus gland (*b*). Arrows indicate points of contiguity of tumor with hyperplastic reticulum cells of thymus tissue. Hematoxylin and eosin; × 30.

Fig. 6.—High-power magnification of the thyroid gland in a male rat treated with thiourea for 14 months. Note the overactivated character of the follicles caused by the goitrogen and compare with the section of the thyroid-like nodule illustrated in Figure 7. Hematoxylin and eosin; × 265.

Fig. 7.—High-power magnification of the thymic adenomata (goiter) shown in Figure 4. Note similarity of this tissue to that of the thyroid gland illustrated in Figure 6. Hematoxylin and eosin; × 320.

densely staining vesicles prominently located at its circumference. These thyroid tumors measured 2 to 4 mm. in their greatest diameter. There was no correlation between type of thyroid neoplasm and the sex of the individual. Since metastases were not found in the lungs of these rats, such thyroid neoplasms were apparently benign. They will be fully discussed in a separate communication.

Although a high correlation existed between the number of thymic and thyroid tumors in our animals, the reciprocal relationship was not absolute. Of the nine rats in the experimental series with thymic adenomata, four occurred in the absence of any frank demonstrable thyroid neoplasia. On the other hand, 6 of the 11 rats possessing neoplasms in the thyroid gland did not have tumors in the thymus.

COMMENT

According to Lahey, Hare, and Warren,[24] many of the distinguishing features of malignancy may be observed even in the normal thyroid gland. Although the present investigation does not support this point of view for the rat thyroid, the high correlation between the number of thymic and thyroid neoplasms encountered in this study suggests that some thymic nodules may represent metastatic thyroid tissue. However, not only did such thymic tumors resemble typical accessory thyroid tissue but the adenomata of the thyroid gland in these animals appeared histologically benign. Furthermore, the thyroid gland tumors under discussion were similar to certain tumors of the thyroid found by Money and Rawson [25] following prolonged administration of thiouracil. These investigators also failed to find evidence of metastases in the lungs of their experimental animals.

It is possible, however, that such experimental thyroid neoplasms have a low grade of malignancy. Metastases commonly assigned to some thyroid gland tumors appear as benign growths or as normal thyroid tissue. They not only simulate normal acinar tissue but also show a greater tendency for physiologic activity (McGavack [11]). The goiters induced in thymus tissue of our experimental rats exhibited similar features. If, indeed, we are dealing with metastatic nodules, then the thymus may come to be looked upon as a favorable site for the lodgement of thyroid metastases. Thus, metastatic lesions in thymic tissue, at least in the rat, can no longer be regarded as rare.

The mechanism of such metastases is questionable. Although a direct lymphatic connection may exist between the thyroid gland and the thymus without the interposition of lymph nodes, as suggested by Williamson and Pearse,[26] such a vascular connection was not demonstrable in animals studied by Chouke, Whitehead, and Parker.[27] Injection studies designed to clarify this point should be carried out in the rat.

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24. Lahey, F. H.; Hare, H. F., and Warren, S.: Carcinoma of the Thyroid, Ann. Surg. **112**:977-1005, 1940.

25. Money, W. L., and Rawson, R. W.: The Experimental Production of Thyroid Tumors in the Rat Exposed to Prolonged Treatment with Thiouracil, Cancer **3**:321-335, 1950.

26. Williamson, G. S., and Pearse, I. H.: Anatomy (Comparative and Embryological) of Special Thyroid Lymph System, Showing Its Relation to Thymus with Some Physiological and Clinical Considerations That Follow Therefrom, Brit. J. Surg. **17**:529-550, 1930.

27. Chouke, K. S.; Whitehead, R. W., and Parker, A. E.: Is There a Closed Lymphatic System Connecting the Thyroid and Thymus Glands? Surg., Gynec., & Obst. **54**:865-871, 1932.

Finochietto and Luchetti [28] have called attention to the occurrence of so-called thyrothymic tracts or laminae of thymic tissue connecting the inferior pole or isthmus of the thyroid gland to the thoracic thymus. These authors state that these pathways are conspicuous in human beings with exophthalmic goiter and that they have a particular technical and therapeutic importance. They maintain that such anatomical connections ("rests") facilitate the descent of cell masses from the thyroid gland which are responsible for endothoracic goiter. No such thyrothymic interconnections, however, have been encountered in the experimental animals used for this study. It is unlikely that thyroid metastases (by emboli or extension) adequately explain the presence of thymic goiters in these rats.

The possibility that thyroid tissue may develop from thymic parenchyma is not wholly conjecture. In 1935, de Winiwarter [29] expressed the opinion that potentialities for thymic and parathyroid tissue were broadly resident in the pharyngeal region. He believed that parathyroid tissue could come from the thymus and the thymus from parathyroid or thyroid tissue. Presumably there is as much reason to believe that thymic tissue can occasionally produce thyroid tissue as there is to believe that thyroid tissue may give rise to thymus. This concept is further strengthened by the recent observation that accessory thyroid tissue may occasionally develop from the medulla of thymus IV in the adult baboon (Van Dyke [30]). Circumstantial evidence indicated that this spontaneous accessory thyroid mass was probably derived from ultimobranchial tissue associated with a thymus IV which was left outside the thyroid gland proper during embryonic development. However, the epithelial component of the thymus could not entirely be dismissed as a possible source for this "new-growth."

If ultimobranchial tissue is, or under certain circumstances can be, the source of accessory thyroid tissue or adenomata (Van Dyke [31]), it should be appreciated that remnants of this tissue may occur in the thoracic thymus of rats. In this animal the anlagen for the thymus and the ultimobranchial body are also derived from one and the same embryonic source (pharyngeal pouch III in the rat). Consequently, ultimobranchial tissue might be expected to persist usually near the cranial pole in the thymus of some rats. Here, as in the case of ultimobranchial tissue normally incorporated within the thyroid gland proper, this tissue may reflect a need for additional thyroid hormone by the development of accessory thyroid nodules (or tumors) following certain growth-promoting stimuli. In view of an apparent reciprocal relationship between the thymus and ultimobranchial tissue, however, it would be difficult to assign such thyroid-like nodules in the thymus to either thymic

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31. Van Dyke, J. H.: Behavior of Ultimobranchial Tissue in the Postnatal Thyroid Gland: The Origin of Thyroid Cystadenomata in the Rat, Anat. Rec. **88**:369-391, 1944; Behavior of Ultimobranchial Tissue in the Postnatal Thyroid Gland: Epithelial Cysts, Their Relation to Thyroid Parenchyma and to "New-Growths" in the Thyroid Gland of Young Sheep, Am. J. Anat. **76**:201-251, 1945; Factors Influencing Preneoplastic Manifestations in Mammalian Thyroid Glands, Scientific Proceedings, Cancer Res. **10**:246, 1950.

epithelium or associated ultimobranchial tissue. Concerning this relationship in the thymus IV of the cat, Mason [32] stated, "As that part of the ultimobranchial body and thymus IV which is not cystic is thymic, cyst formation and thymic transformation are in reciprocal relation."

The evidence in the present study, therefore, has been interpreted as indicating that these thymic tumors represent essentially accessory thyroid tissue which has become goitrous following administration of thiourea. Presumably, such goiters, in response to an excessive demand for thyroid hormone, arise by hyperplasia under the influence of an increased output of thyrotropic hormone. In this respect they are comparable to the thyroid parenchyma in the neck. Since these mediastinal (thymic) goiters may be multiple, it is probable that they are derived from "cell rests" dislodged from the thyroid gland during the growth mechanics of embryonic development.

However, because such "cell rests" are not readily distinguishable normally as a separate entity from the epithelial component of the thymus, the thymus gland cannot be completely discarded as a potential extrathyroidal source for the thyroid hormone following adequate stimulation.

Under the conditions of this experiment, the present findings indicate that embryonic thyroid "rests" associated with the thymus gland are apparently numerous in the rat. The potentialities for the development of accessory thyroid tissue or aberrant goiters in the mediastinum, at least in this mammal, are, therefore, considerable and worthy of recognition.

Further investigations designed to help clarify the evident reciprocal relationship between the thyroid gland and the thymus during thyrotoxicosis in the human being are in progress. However, certain factors which influence the thymus gland, as well as the ultimobranchial tissue component of the thyroid gland in the rat, will be considered shortly in a separate publication.

SUMMARY AND CONCLUSIONS

Thymic adenomata resembling accessory thyroid tissue (goiters), some of which appear to be incipient, are reported in a large number of Wistar rats following prolonged administration of thiourea. Although these neoplasms frequently occurred coincidentally with tumors in the thyroid gland, little similarity existed between "new-growths" in the two sites.

The possibility that such mediastinal tumors (goiters) represent metastases from the thyroid gland is considered unlikely but is discussed. The concept that they may be derived from ultimobranchial tissue carried into the thymus during embryonic development, or even from hyperplastic foci of thymic epithelium, has been suggested and is also briefly discussed.

The evidence at hand, however, has been interpreted as favoring an origin from embryonic thyroid "rests." Such nodules probably develop as compensatory aberrant goiters following excessive thyrotropic hormone stimulation caused by the goitrogen. In the rat at least, this study indicates that the potentiality for accessory thyroid tissue or aberrant goiters in the mediastinum is great.

32. Mason, V. A.: The Fate of the Ultimobranchial Body in the Cat (Felis Domestica), with Special Reference to Cyst Formation Within the Thyroid, Am. J. Anat. **49**:43-63, 1931.

DEVELOPMENT OF PIGMENTARY CIRRHOSIS IN COOLEY'S ANEMIA

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DEPOSITION of iron in the reticuloendothelial system, in the peripheral zone of the liver lobule, and in the kidneys has long been a well-recognized anatomical feature of the hemolytic anemias. In these conditions, the lysis of red blood cells is premature, precipitate, and profound and transcends the physiologic controls of hemoglobin metabolism with the temporary accumulation of hemosiderin in tissue depots of disposal. But in Cooley's anemia, although a hemolytic component is present due to destruction of imperfect erythrocytes, the pattern of iron deposition is cumulative and singularly distinctive.

In this disease, Bradford and Whipple [1] noted the remarkable constancy of iron pigment deposition in the entire liver lobule, myocardium, pancreas, adrenal gland, and abdominal lymph nodes and the comparative freedom of the spleen, marrow, and kidneys from pigment deposition. Although this anatomical distribution of iron pigment was similar to that seen in idiopathic hemochromatosis, the conventional manifestations of idiopathic hemochromatosis, such as cirrhosis and "bronzed diabetes," were lacking.

Because of current interest in the pathogenesis of hemochromatosis, this report of a case of a patient suffering from Cooley's anemia for 13 years but sustained through the infusion of 225 blood transfusions is of value. It illustrates the effects of iron overload upon tissue "already burdened by some fundamental change in the method of handling blood pigments." [1]

REPORT OF A CASE

Clinical History.—J. M., the only child of Sicilian parents, first seen at the age of 18 months, was brought by her parents to the family doctor, as she appeared weak and puny for her age. These clinical findings had been present for about one year but had increased in severity during the previous two to three months. The family doctor palpated a large spleen and referred her to the hospital.

Birth and Family History.—She was born at full term after an uneventful pregnancy and delivery. For the first three days of life, she was breast fed and afterwards placed on a formula. Vitamins were given in the usual dosages, and early development was apparently normal.

Both parents were alive and healthy. After the child had been under study for some time, a first cousin was seen suffering from the same disease.

Physical Examination.—She was a puny, irritable child with a skin described as being of a café au lait color. A generalized lymphadenopathy was discovered, all nodes being small, firm, and discrete. No abnormalities of the cardiovascular or pulmonary systems were discovered.

From the Department of Pathology, St. Louis University School of Medicine.

1. Whipple, G. H., and Bradford, W. L.: J. Pediat. **9**:279, 1936.

The abdomen was protuberant, due to a large spleen with a smooth surface and sharp borders. The spleen extended from the left flank to the lateral border of the left rectus muscle, with its lower limit being 1 in. (2.5 cm.) above the symphysis pubis. The liver was palpable 1 in. below the right costal margin and had a smooth contour. No abnormalities of any other system were noted.

Investigations.—Details of hematologic studies during the course of the disease are given in Table 1.

Blood Chemistry.—On first admission to the hospital, blood sugar was 110 mg. per 100 cc. of blood; uric acid 3.5 mg. per 100 cc.; cholesterol 374 mg. per 100 cc.; phosphorus 4.8 mg. per 100 cc.; calcium 9.1 mg. per 100 cc.; icterus index 12; van den Bergh's test, indirect positive 0.9 mg., direct negative. Serologic reactions by the Kahn and Kline methods were negative on this and later admissions.

Urine.—Urinalysis showed no albumin, sugar, or acetone; 2 white blood cells per high-power field.

TABLE 1.—*Résumé of Hematologic Findings*

Date Admission	Admission	Age, Yr.	Red Cells per Cu. Mm.	Hemoglobin, Gm. per 100 Cc.	White Cells per Cu. Mm. (Corrected)	Nucleated Red Cells per 100 WBC	Target Cells	Reticulocytes, %	Red Cell Fragility, %	Blood Transfusion Total to Date, Cc.	Anisocytosis	Poikilocytosis	Polychromatophilia	Comments
10/ 8/40	2	5/6	2,480,000	4.2	31,750	30	+	..	0.36 to 0.36	875	++	++	++	Platelets 33,510; jaundiced; icterus index 12
10/ 6/42	16	3	2,560,000	6.0	8,550	23	+	8	0.44 to 0.30	12,005	++	++	++	Splenectomy
3/18/43	17	3	2,400,000	5.8	12,000	710	+	..	0.44 to 0.10	12,900	++	++	++	Persistent rise in nucleated red cells following splenectomy
4/12/44	28	5	2,210,000	6.2	61,800	87	+	10		16,510	++	++	++	.....
2/ 3/48	49	9	2,390,000	8.0	15,800	311	+	..		37,350	++	++	++	.....
3/15/50	64	11	3,450,000	8.0	20,000	81	+	..	0.40 to 0.20	53,260	++	++	++	.....
5/ 7/52	78	13	2,920,000	7.1	17,700	554	+	..	0.44 to 0.28	68,730	++	++	++	.....

X-Rays.—Although the skull showed no alterations, the long bones revealed loss of lime salts and an extremely thin granular cortex. A chest plate revealed definite cardiac enlargement, both to the right and to the left.

Treatment and Course in Hospital.—Iron salts by mouth, liver extracts parenterally, and blood transfusions were given on this and subsequent admissions. The response to treatment was equivocal. On discharge from the hospital following the first admission, a blood count showed red blood cells 5,650,000 per cu. mm.; hemoglobin 12 gm. per 100 cc.; white blood cells 14,500 per cu. mm., with a normal differential count and a reticulocyte count of 0.1%.

Interval History.—At first the child gained appetite and weight. She learned to walk. After about two months, progressive anorexia with weight loss, increasing weakness, fatigability, and constipation were noted. This established the future pattern of the disease, which necessitated hospitalization every six to eight weeks for the rest of her life. On subsequent admissions the physical findings remained unchanged, except that occasionally she was slightly jaundiced.

Subsequent Investigations.—Pertinent investigations included prothrombin time 24 seconds; bleeding and clotting time, both one minute. Clot retraction began at 25 minutes and was completed in three hours. The sedimentation rate was usually raised. Serum proteins amounted to 6.0 gm. per 100 cc., albumin 4.2 gm. per 100 cc. and globulin 1.8 gm. per 100 cc. Howell-Jolly bodies and target and stipple cells were frequently recorded. Volume index was 0.6, and volume of packed cells was 13.8 cc. It was noted that immature red cells were present up to 84% prior to transfusion, and following transfusion these showed an immediate fall. For blood counts,

see Table 1. The urine and feces always contained increased amounts of urobilinogen. Repeated x-rays of the small and long bones were taken to determine the presence of typical bony changes. However, apart from granularity of the cortices with loss of lime salts, the results were negative, except for a plate of the hands and feet which showed a very coarse appearance of the bony trabeculae in the small bones. An electrocardiogram in 1945 showed evidence of myocardial change.

On account of unremitting disease, a splenectomy was carried out on Nov. 12, 1942 (child was three years of age).

Report on Pathological Specimen.—The spleen weighed 286 gm. It had a smooth red-brown capsule, and on cut section the parenchyma was firm, and the Malpighian bodies prominent. Four small lymph nodes were also removed. Microscopic examination showed a fine diffuse fibrosis. Within the sinusoids, numbers of myelocytes were seen with a few megakaryocytes and nucleated red cells. Small amounts of golden yellow pigment granules within macrophages and also entrapped within the trabeculae were seen. The Malpighian bodies, although active, were unremarkable. The lymph nodes, apart from fairly heavy localized deposits of golden yellow pigment, were unremarkable.

No clinical improvement was noted following splenectomy; the anemia progressed and remained refractory to treatment as before. The blood counts now showed a persistent rise in nucleated red blood cells. Following the operation, the liver progressively enlarged until at time of death it was palpable at the pelvic brim.

This unfortunate child's further history was characterized by steady deterioration through the years. Blood became increasingly difficult to cross match; minor reactions to transfusions became increasingly common. At the final admission, the child was desperately ill in heart failure, with effusions in all serous cavities, refractory to all efforts of treatment, and she died on May 13, 1952. In all, she received 225 pints of blood during her lifetime, much of which was in the form of concentrated red cells.

Autopsy (SMH A52-63).—Autopsy was performed eight hours after death on the body of this 13-year-old American-Sicilian girl. The body was markedly emaciated. Apart from slight dusky pigmentation around the axilla, there was no discoloration of the skin or sclerae. The abdomen was slightly distended, and the lower limbs showed moderate pitting edema. There was a midline hypogastric incision 11 cm. in length extending from the costal margin to the umbilicus, the site of splenectomy.

Body Cavities: Each pleural cavity contained 300 cc. of clear, yellow, serous fluid. The pericardial cavity contained 150 cc. of similar fluid. Within the peritoneal cavity, 500 cc. of clear yellow fluid was measured. In the left upper quadrant of the abdomen, firm omental and visceral adhesions to the diaphragm and lateral gutter walls were noted.

Heart: The heart weighed 450 gm. The valvular orifices were free of abnormalities. The coronary arteries were patent throughout. Transverse section through the interventricular septum showed diffuse streaks of scar tissue, and the heart muscle was an ochre brown color. Dipping the heart muscle into a prepared solution of potassium ferrocyanide and hydrochloric acid elicited a deep blue color (Fig. 1 *B*).

The combined weight of the lungs was 450 gm. In the basilar and posterior portions of both lungs, there were scattered regions of blue-red hypocrepitant, atelectatic lung tissue. The bronchial tree and the pulmonary arteries and veins were patent and free of lesions throughout their distributions.

Gastrointestinal Tract: In both the stomach and the small bowel, particularly in the ileum, there was a tan-brown discoloration of the mucosa not due to postmortem staining. The remaining layers of the gut wall were without alteration or pigmentation.

Lymph Nodes: All lymph nodes examined—cervical, inguinal, hilar, para-aortic, and mesenteric—were deeply pigmented, remarkably rust colored in appearance, and firm in texture. The mesenteric and periportal lymph nodes showed this distinctive coloration to the greatest extent.

Liver: This organ weighed 2,250 gm. and filled the entire upper right and left quadrants of the abdomen. There was a diffuse nodularity of the surface, and the liver substance showed throughout a distinctive ochre brown coloration. Both lobes shared equally in pigmentation and nodularity; both of these gross alterations were distinct, diffuse, and uniform. On application of potassium ferrocyanide and dilute hydrochloric acid, the liver parenchyma became a deep blue color. The intrahepatic bile ducts were patent and not dilated.

Pancreas: The pancreas weighed 150 gm., was diffusely indurated, and was red-brown rusty color (Fig. 1*A*). The lobules of pancreatic tissue were demarcated by thick, prominent, white, intervening scar tissue. This diffuse alteration was particularly noticeable after application of potassium ferrocyanide and hydrochloric acid, which resulted in a deep blue color of the parenchyma, the whitish scar tissue standing out in contrast.

Kidneys: Each kidney weighed 120 gm. No external abnormalities were noted. On cut section, however, the usual color zones were obscured by deposits of the red-brown pigment. Both kidneys were of normal size and shape.

Skull and Central Nervous System: No gross changes were observed in the bony tables of the skull.

The brain showed no external pigmentation; however, the choroid plexus was a pronounced red-brown color. The pituitary gland showed rusty pigmentary changes.

Bone Marrow: Inspection of the vertebral bone marrow showed this to be a uniform diffuse purple color.

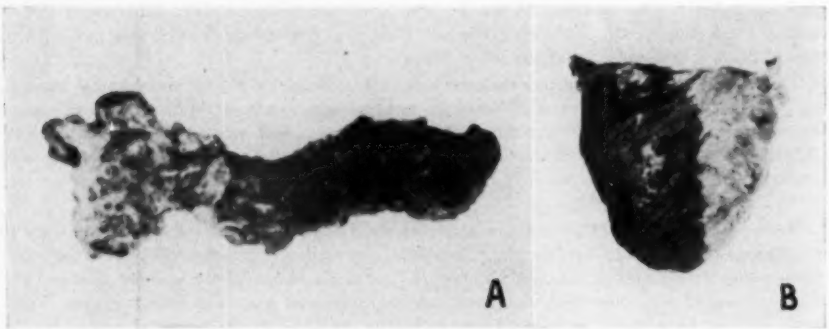

Fig. 1.—*A*, gross specimen of pancreas showing broad bands of scarring and body and tail of organ stained by potassium ferrocyanide-hydrochloric acid giving intense coloration (black in photograph). *B*, gross photograph of interventricular septum of heart, with one-half revealing consummated Prussian blue reaction.

HISTOPATHOLOGIC EXAMINATION

Brain, choroid plexus, liver, lungs, kidneys, heart, pancreas, gastrointestinal tract, adrenals, thyroid, uterus, Fallopian tubes, ovaries, pectoral muscle, knee joint synovial tissue, aorta, bone marrow, and lymph nodes were prepared and stained by hematoxylin and eosin, Masson's trichrome stain, and potassium ferrocyanide hydrochloric acid. Granick's cadmium sulfate technique [2] for the demonstration of ferritin was also attempted on liver and gut. In the brain and related structures, hemosiderin, without the accompaniment of scarring, was found in the choroid plexus, being present as fine intraepithelial granules. The liver showed intense iron accumulation with the parenchymal cells and associated with a diffuse and intense fibrosis, creating a multilobular pattern (Fig. 2*A*). There was no evidence of recent cytolysis or inflammation. The bile ducts were unaltered in form and pattern and

2. Granick, S.: Bull. New York Acad. Med. **25**:403, 1949.

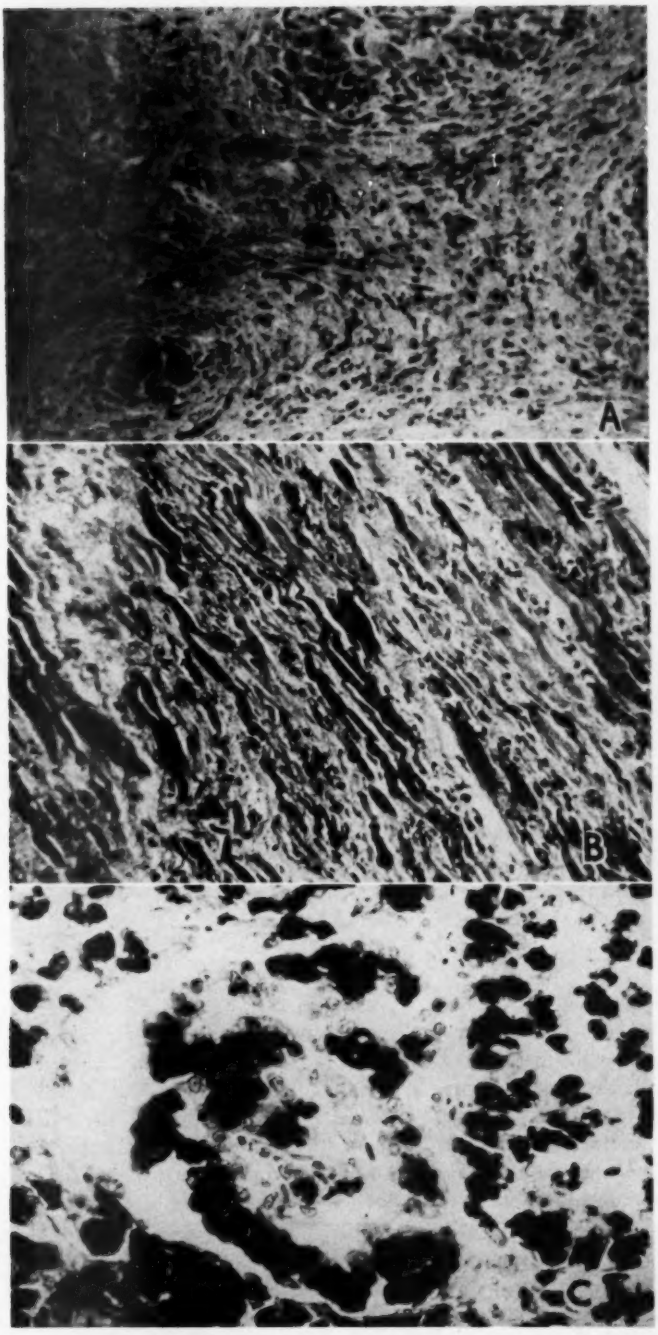

Fig. 2.—*A*, liver showing extensive fibrosis and iron pigmentation. Hematoxylin and eosin; × 210. *B*, heart showing intense iron pigmentation (black) and fragmented myocardial fibers. Considerable scarring also noted. Prussian blue; × 210. *C*, islet of Langerhans showing profound iron pigmentation. Pancreatic acini almost obliterated by accumulation of iron. Prussian blue; × 210.

were free of any accompanying infiltration of leucocytes. In the zona glomerulosa and fasciculata of the adrenal and thyroid glands, there were a few cells charged with hemosiderin. No diffuse scarring was noted.

The kidney showed deposits of hemosiderin in the convoluted and collecting tubules. There were no structural alterations in the glomerular vascular or interstitial tissues.

Uterus, Fallopian tubes, ovaries, muscle, and synovia were negative for the Prussian blue reaction and free of a fibrotic response. Bone and bone marrow showed profound erythroblastic hyperplasia. In sections from the sternum and vertebrae there were scattered foci of new bone formation with prominent dominant osteoblasts. Little iron pigmentation was encountered. Within the lymph nodes, colonial myeloid metaplasia was observed. Well-defined Gamma-Gandy nodules were found in many of the lymph nodes. These nodules consisted of masses of calcium and iron agglomerated together. Granules of iron, intracellular and extracellular, were diffusely scattered throughout the cortical and medullary portions of the lymph nodes.

Fresh tissue from liver and duodenum was subjected to cadmium sulfate precipitation, but we were unable to demonstrate the crystals of ferritin.

The lungs were free of iron pigmentation and showed only basal protein-poor fluid within the alveoli.

In the heart there was extensive myocardial fiber disruption and a diffuse distribution of hemosiderin distorting the muscle fibers (Fig. 2*B*). There was a remarkable amount of fresh and old fibrosis, and in some of the scarred areas there was entrapped Prussian blue pigment. Within the pancreas, like the heart and liver, there was an intense accumulation of iron pigment. This pigment was equally abundant within the acinar and insular elements. The fibrosis of the pancreas was both fresh and old and diffusely distributed throughout this gland (Fig. 2*C*).

In the gastrointestinal tract there was intramucosal pigment accumulation which was particularly intense in the duodenum and jejunum.

The skin from the axilla showed a few granules of iron around the sweat glands. There was no increase of melanin in the epidermis.

COMMENT

Although Cooley,[3] Wollstein,[4] and other investigators[5] noted the presence of granules of iron in the liver, it remained for Whipple and Bradford to note the similarity of pigment distribution in Cooley's anemia and conventional hemochromatosis. In the classic reports by Whipple and Bradford, the distribution of the pigment in the liver, pancreas, endocrine organs, skin, and heart muscle (usually regarded as preferential sites in conventional idiopathic hemochromatosis) was recorded, but there was no evidence of fibrosis in the liver, pancreas, or myocardium and no other manifestations of idiopathic hemochromatosis.

In this report of a case, diffuse hepatic fibrosis was profound, and equally profound was the deposition of iron in the liver. In addition to this pigmentary

3. Cooley, T. B.; Witwer, E. R., and Lee, P.: Am. J. Dis. Child. **34**:347, 1927.
4. Wollstein, M., and Kreidel, K. V.: Am. J. Dis. Child. **39**:115, 1930.
5. Williamson, G. R., and Crawford, R.: New Orleans M. & S. J. **98**:280, 1945.

cirrhosis, there was intense deposition of hemosiderin in the islets of Langerhans and pancreatic acini, with associated prominent scarring. Despite this intense accumulation of pigment in the insular tissue, there was no clinical evidence of diabetes mellitus. Although no morphologic quantitative observations were made on the postmortem material, apparently a sufficient reserve of islet tissue remained to prevent diabetes. Bronzing of the skin was minimal, consisting of small accumulations of iron pigment around the dermal sweat glands. Microscopic examination of the myocardium showed a heavy and diffuse deposition of iron pigment within the myocardial fibers; considerable disintegration of these fibers, together with much fibrosis, was seen. These two lesions were probably responsible for the terminal heart failure accounting for the hydropericardium, hydrothorax, ascites, and leg edema. It is to be noted in this connection that in idiopathic hemochromatosis heart failure is the cause of death in 10% of all cases.[6]

Chemical analysis of the liver, pancreas, and myocardium revealed the total amount of iron in these three organs to be 19.34 gm. and with the partitional analysis as shown in Table 2.

TABLE 2.—*Chemical Assay of Viscera*

	Iron in Aliquot, %	Organ Weight, Gm.	Iron Content (Absolute), Gm.
Liver	0.814	2,250	18.3
Pancreas	0.492	150	0.5
Heart	0.120	450	0.54

TABLE 3.—*Chemical Assay of Viscera (Compiled from Whipple and Bradford)*

	Age, Yr.	Transfusions	Weight Liver, Gm.	Analysis, Gm./100 Gm.	Absolute, Gm.
Case 3	5	2,020 cc.	810	0.292	2.3
Case 4	7	None	1,175	0.114	1.3
Case 5	7	"Several transfusions"	2,550	0.395	10.0

Control studies were carried out on three young women dying from sudden traumatic deaths (St. Louis County Hospital medical legal cases) which indicated that the total iron present in these three organs gave values between 0.30 and 0.34 gm. Thus a marked difference existed between these "controls" and this repeatedly transfused example of Cooley's anemia.

In view of the fact that 54 gm. of iron were infused during life and only 19.3 gm. were present in tissue stores at death, heavy utilization drafts upon iron stores must have occurred in this case; if one considers the supplemental ferrous salts obtained through the gut, the total iron intake during life was probably much greater than 54 gm.

The only example in the literature in which chemical analyses can be compared is between the present example and Case 5 in Whipple and Bradford's series of fatal Mediterranean anemia.

The pigment distribution within the liver lobule in Whipple's Case 5 and in our example appeared to be morphologically similar but without cirrhosis in his Case 5.

6. Sheldon, J. H.: Haemochromatosis, London, Oxford University Press, 1935.

In Bradford's case the magnitude of iron within the liver, without benefit of blood transfusion, is of almost the same magnitude as in the case reported here. It is our postulate that the common denominator is related to one factor: the prolongation of life above and beyond the usual span in the natural course of this disease; the multiple blood transfusions led to greater longevity of life, thus fostering recurrent or protracted episodes of hypoxia.

The total amount of iron estimated in the liver, pancreas, and myocardium was 19.34 gm.; in many reported examples of transfusional siderosis the iron content is much greater. In this repeatedly transfused example of Cooley's anemia, extreme parenchymal fibrosis was found in the liver, pancreas, and heart; whereas in transfusional siderosis, diffuse cirrhosis, pancreatic fibrosis, and pigmentary cirrhosis, myocardial scarring is rare.[7] On morphological grounds, it is our belief that the pattern of pigment deposition in this case was distinguishable from transfusional siderosis but indistinguishable from idiopathic hemochromatosis. Consideration of all of these distinctions strengthens the concept that some factor or factors, other than mere iron overload, was needed for the development and progression of parenchymal fibrosis.

From our previous studies on transfusional siderosis and exogenic hemochromatosis,[7b] it is probable that iron alone is incapable of evoking diffuse fibrosis. It has been shown that lowering of oxygen tension may bring about increased iron storage.[8] Racker and Krimsky[9] have demonstrated that relatively high concentrations of ferrous salts inhibit the triose phosphate dehydrogenase enzyme system. The recurrent, nonresponsive bouts of anemia may, through oxygen deprivation, affect hepatic or other cells with a high metabolic level, inhibiting their enzyme systems, resulting in degenerative cellular changes and consequent fibrosis. In the liver, the pattern of cirrhosis is identical to that observed in diffuse "nutritional" cirrhosis (Laennec type) and unlike the reported examples of postnecrotic fibrosis alleged to follow viral hepatitis. It is readily granted that the possibility of infectious hepatitis following blood transfusion could have occurred, but interpretative morphology indicated that the liver fibrosis developed *pari passu* with hemosiderin accumulation. In this respect, the pathogenesis of the liver fibrosis in exogenic hemochromatosis would be different on temporal grounds from that which Muir and Dunn believed[10] causes cirrhosis in conventional hemochromatosis.

In utilizing this case for the development of the discussion on pigmentary cirrhosis in Cooley's anemia, two cardinal points emerge.

First, the deposition of pigment and its accumulation are just as typical of Cooley's anemia as are the factors of inheritance, the dystrophic red cell or the disturbed bone growth.

Second, this example of pigmentary cirrhosis with skin pigmentation and pancreatic fibrosis following multiple transfusions in Cooley's anemia offers a link in the understanding of exogenic hemochromatosis. On the basis of the quantitative

7. (*a*) Schwartz, S. O., and Blumenthal, S. A.: Blood **3**:617, 1948. (*b*) Wyatt, J. P.; Mighton, H. W., and Moragues, V.: Am. J. Path. **26**:883, 1950.

8. Hahn, P. F.; Bale, W. F.; Ross, J. F.; Balfour, W. M., and Whipple, G. H.: J. Exper. Med. **78**:169, 1943.

9. Racker, E., and Krimsky, I.: J. Biol. Chem. **173**:519, 1948.

10. Muir, R., and Dunn, J. S.: J. Path. & Bact. **19**:417, 1915.

iron analysis and from previous observations, it is our postulate that progressive parenchymal fibrosis encountered in this exogenic hemochromatosis is related to the continuing hypoxia or an allied mechanism, rather than to the cellular accumulation of iron.

SUMMARY

The characteristic accumulation of iron in Cooley's anemia is emphasized, as this visceral distribution of pigment simulates that observed in idiopathic hemochromatosis. With the advent of multiple transfusions as a therapeutic measure in Cooley's anemia, the complete picture of classical hemochromatosis with the pathologic alterations of pigmentary cirrhosis and pigmentation of the skin, myocardium, and pancreas has been recorded.

From chemical, morphological, and temporal studies, it is suggested that the development of these distinctive pigmentary lesions with fibrosis is due to continuing or episodic tissue hypoxia, rather than excessive iron storage accumulating from multiple blood transfusions.

Case Reports

SMALL FORMS OF BLASTOMYCES DERMATITIDIS IN HUMAN TISSUES

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IN NORTH American blastomycosis, the double-contoured budding yeast forms seen in infected tissue usually range in diameter from 8μ to 15μ.[1] They incite lesions that exhibit suppurative, necrotic, and granulomatous inflammation with numerous foreign body giant cells. Wade and Bel [2] and Manwaring [3] each reported an unusual case in which small forms of the micro-organism measuring 2μ to 4μ in diameter were abundant and were found free or clustered in macrophages. Manwaring called attention to this feature as simulating histoplasmosis. Three similar cases were recognized by Schwarz and Baum.[4] In the case herein reported, small forms were seen in lung lesions, and Blastomyces dermatitidis was recovered culturally and on animal inoculation.

REPORT OF A CASE

A 69-year-old man in good general health, a native of New York State, had an ischiorectal abscess while in Florida in May, 1951. An operation, details of which are not available, was undertaken, apparently for drainage. The patient returned to his home in New York feeling ill and entered a hospital June 11, 1951, with a temperature of 101 to 102 F., loss of weight, and mental confusion. He died after a four-day illness. During the hospital stay, a roentgenogram showed changes in both lungs suggestive of silicosis and an area of increased density in the lower left lobe. Candida albicans and a member of the coliform group were isolated from the ischiorectal abscess. Results of agglutination tests on clotted blood were noncontributory.

Postmortem examination, limited to the chest, disclosed a hard knotty area in the middle lobe of the left lung and a large abscess involving pleura but apparently extending into the right lobe of the liver. The abscess or empyema contained approximately a pint of yellowish fluid. Formalin-fixed lung tissue and swabs, films, and cultures of the abscess exudate were submitted for examination.

Histologic examination of the portion of lung submitted showed exudative and granulomatous lesions. The exudative sites consisted of necrotic areas containing large numbers of neutrophiles and budding fungi, most of the fungi being free. The granulomatous lesions consisted of dense fibrous tissue infiltrated with numerous

From the Division of Laboratories and Research, New York State Department of Health.

1. Manual of Clinical Mycology, prepared for National Research Council, Division of Medical Sciences, by N. F. Conant, D. S. Martin, D. T. Smith, R. D. Baker, and J. L. Callaway, Philadelphia, W. B. Saunders Company, 1945, p. 36.

2. Wade, H. W., and Bel, G. S.: A Critical Consideration of Systemic Blastomycosis: With Notes on Certain Special Features and Report of 5 Cases, Arch. Int. Med. **18**:103-130, 1916.

3. Manwaring, J. H.: Unusual Forms of Blastomyces Dermatitidis in Human Tissues, Arch. Path. **48**:421-425, 1949.

4. Schwarz, J., and Baum, G. L.: Blastomycosis, Am. J. Clin. Path. **21**:999-1029, 1951.

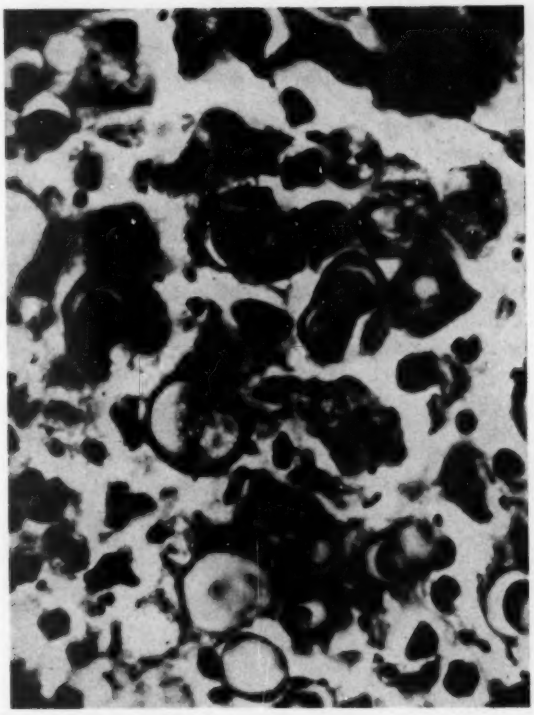


Fig. 1.—Blastomyces dermatitidis of average size lying free and in phagocytic cells. A few small forms are seen (lung); × 1,300.

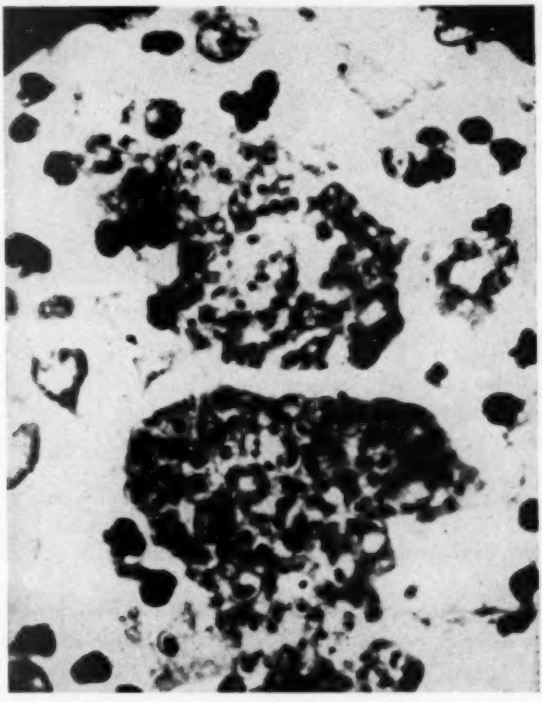


Fig. 2.—Small forms (2μ to 5μ) of Blastomyces dermatitidis in large mononuclear phagocytic cells resembling Histoplasma capsulatum (lung); × 1,300.

mononuclear cells and foreign body type giant cells which varied from few to many in different areas. Fungi were found free and in the large phagocytic cells (Fig. 1). Near these granulomatous foci, large phagocytic mononuclear cells were filled with large numbers of small forms of the fungus (Fig. 2). At this time, a diagnosis of a mixed mycotic infection with B. dermatitidis and Histoplasma capsulatum was entertained. Careful scrutiny, however, revealed that the small forms only superficially resembled H. capsulatum and that they were more likely small forms of B. dermatitidis. This conclusion was based on budding forms of small size and on a continuous series of micro-organisms ranging from small to large. Because lung

Fig. 3.—Section of hamster testis showing large forms of Blastomyces dermatitidis; × 1,300.

sections showed fungi, the blood previously submitted for agglutination tests was reexamined and the clots were cultured on Sabouraud's and blood agar. Only colonies typical of B. dermatitidis of classical size were isolated. Subcultures of colonies were also of classical size. Acid-fast bacilli were found in direct films from the pleurohepatic abscess, and culturally typical Mycobacterium tuberculosis was recovered, which proved virulent for guinea pigs. Numerous sections of the human lung were stained by the Ziehl-Neelsen method, but no acid-fast bacilli were found. Tuberculosis may have preceded this lesion or coexisted in portions of lung not available for study.

Sections of hamster testes injected with material from the pleurohepatic abscess showed large forms of B. dermatitidis (Fig. 3) and anatomic tuberculosis in which

acid-fast bacilli were demonstrated. Guinea pigs injected with similar material died of tuberculosis, and no fungi were seen in the lesions. Typical Myco. tuberculosis was recovered culturally from the hamster testes.

COMMENT

This case demonstrates the variety of histologic pictures that can be seen in North American blastomycosis. The large forms can be found free or in foreign body giant cells; the small forms can also be found free but when phagocytized are usually packed in macrophages. It is less common to see the large forms in macrophages or the small forms in foreign body giant cells. The histologic pattern of phagocytosis is apparently conditioned by the size of the micro-organism. Thus the picture suggestive of histoplasmosis is developed in response to micro-organisms in the size range 2μ to 5μ or approximately in the range of the H. capsulatum. The clue to the diagnosis lies in the parasitized macrophages, wherein close examination reveals at least one moderately large micro-organism with a double-contoured wall or some small micro-organisms showing single buds (Fig. 2).

Why does the fungus develop these small forms? It may be the result of rapid multiplication due to increased susceptibility of the host. The rapid course of the clinical illness suggests this. In the present case, and also that of Wade and Bel, the patients had tuberculosis, and in Manwaring's case the patient had carcinoma of the gall bladder. All three patients were debilitated. Manwaring reported that ether anesthesia was administered during operation, five days prior to death, and he believed this to be contributory to death. In the present case, a history of similar anesthesia could not be obtained. Small forms in the lung were demonstrated in the present case and in Manwaring's. Small forms have been seen, however, in such other sites as skin, bone marrow, and testes, indicating that it is not an exclusively pulmonary lesion. The possibility that the small forms are due to different strains or to coparasitism is less likely for three reasons: Forms as small as those seen in tissue are unknown in mycology; the small forms in tissue have so far been associated with those of the usual size, and transitions could be seen between the two sizes; finally, the micro-organisms recovered by culture and animal inoculation in this case are classical in size.

SUMMARY

A case of tuberculosis complicated by septicemia with Blastomyces dermatitidis is reported in which small forms of the fungus were conspicuous in the sections of lung. The size, number, and phagocytosis of the fungi in macrophages suggested infection with Histoplasma capsulatum. Minute study showed characteristic budding in the small forms and all gradations in size up to the usual dimensions. B. dermatitidis of classical size were culturally recovered. In animals, lesions produced with exudate and cultures from the patient showed only large forms. The suggestion is made that the histologic picture in blastomycosis is partly dependent upon the size of the micro-organisms and that small size, where it is observed, is a function of host resistance, rather than a peculiar strain of the micro-organism.

News and Comment

Markle Scholar Award for Dr. Penick.—Dr. George D. Penick, Instructor in Pathology, University of North Carolina, has been selected as a Markle Scholar in Medical Science for 1953. Dr. Penick is the second member of the Pathology Department at the University of North Carolina to become a Markle Scholar, Dr. John Graham having been selected in 1949.

Books

The Anatomy of the Nervous System: Its Development and Function. By Stephen Walter Ranson, M.D., Ph.D., Late Professor of Neurology and Director of Neurological Institute, Northwestern University Medical School, Chicago. Revised by Sam Lillard Clark, M.D., Ph.D., Professor of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn. Ninth edition. Price, $8.50. Pp. 581, with 434 illustrations, 18 in color. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

The ninth edition of this well-known book has been revised, as the previous one, by Sam Clark, of Vanderbilt University, who has, on the whole, done an excellent job. When all is said and done, this is probably the best book on neuroanatomy for the more mature student who not only wants to get by but also wants to have a book that will stay with him for many years to come.

Yet there are, as is only natural, a few points which could bear improvement. Chapter 1 practically begins with this sentence: "While the tissue called nervous conducts impulses especially well and is thus said to possess irritability." It neither follows nor is it true. Nervous tissue is actually much less irritable than, e. g., the rods and cones of the retina or the hair cells of the inner ear. These time-honored platitudes might well disappear.

I am not convinced that the account of the development of the septum and the commissures (p. 49) is a very good and truthful one. I agree that the commissura anterior develops in the lamina terminalis, but I am not so sure about the other two. In any event, to state that the anterior commissure and the hippocampal commissure are both rhinencephalic is a bit stiff.

The following chapter on the histogenesis, on the spinal cord, and on the lower centers of the brain with its survey of the cranial nerves is as good as it always was. The chapter on the cerebellum is brought up-to-date and reads well. The reticular substance is not worked so well into the story. It appears only as an appendix on page 372.

I am much less satisfied with the chapter on the thalamus. I do not think much is gained by having the old nomenclature (with Flechsig's nucleus semilunaris) in the older figures and the newer and different nomenclature in the more modern illustrations where the semilunaris appears as the nucleus ventralis posteromedialis, and I cannot see much excuse for giving the elaborate but nonetheless confusing nomenclature adopted by Walker in a long list where among the medial nuclei figure both the medial group and the intralaminar nuclei, particularly when the intralaminar nuclei are singled out for comment afterwards. The chapter on the thalamus deserves to be brought up-to-date once more.

The chapters on the telencephalon are fairly satisfactory, although there are a few points which I should like to raise. The most important is the extent of the rhinencephalon; this is given in its classical extent without taking into account the work of Brodal and some of the modern electrical work which makes it pretty clear, so it seems to me, that the hippocampus is not truly rhinencephalic. One may disagree, but I do not think that one can go past this question in complete silence.

One could improve on the presentation of the neocortex. I would recommend as the first step to eliminate Figure 249. The cortical representation of movements is good; the suppressor zones may not stand up in the long run; cortical autonomic representation could, I think, be

sharpened, particularly after Dell's excellent review in the *Journal de physiologie*. It might have been possible to point at least in passing to the electroencephalogram. The rest (great pathways, clinical applications, and the brain sections) is of the usual excellence.

All these are but minor criticisms of an otherwise excellent book which I personally still prefer to all others.

A Textbook of Clinical Pathology. Edited by Seward E. Miller, M.D., Medical Director, United States Public Health Service; Chief, Division of Occupational Health, Washington, D. C. Fourth edition. Price, $9.00. Pp. 1060, with numerous illustrations. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1952.

This fourth edition of "A Textbook of Clinical Pathology" maintains the high standards of the previous editions. It presents an excellent, modern, and relatively detailed survey of each of the fields of interest to the practicing clinical pathologist, the pathology resident, practitioners and residents in other fields, and the intern and medical student. The general plan of the book is similar to that of previous editions in that each subsection in clinical pathology is written by a recognized expert, the book being the joint product of eight authors. The different sections present basic principles of the laboratory procedures, enough details of techniques to emphasize conditions necessary for accurate results, and an unusually good presentation of the clinical importance of the laboratory procedures in most cases. The bibliography is up-to-date and well chosen. Some of the major changes from the previous edition are (1) the entire hematology section has been consolidated under one authorship (L. W. Diggs), and (2) the sections on clinical chemistry, liver and kidney function tests, parasites, urine, gastric contents, sputum, and cerebrospinal fluid have been completely rewritten by new authors. New sections on immunologic tests, assay of chemotherapeutic and antibiotic agents, and diagnosis of virus and rickettsial disease have been added. The chapter on blood groups is excellent.

The book should be of special usefulness to the pathology resident since it is written in such a fashion as to provide him with the basic material to begin to answer his question, "How much should I know about this particular subfield of clinical pathology to be qualified to begin the practice of clinical pathology in a hospital?" The book should also be of special usefulness to medical students because of its clear presentation of the basic principles of the various types of procedures and their clinical application.

Treatment of Mental Disorder. By Leo Alexander, M.D., Director, The Neurobiological Unit, Division of Psychiatric Research, Boston State Hospital, and Instructor in Psychiatry, Tufts College Medical School, Boston. Price, $10.00. Pp. 507, with 143 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

Ophthalmic Pathology. By Jonas S. Friedenwald, Helenor Campbell Wilder, A. Edward Maumenee, T. E. Sanders, John E. L. Keyes, Michael J. Hogan, W. C., and Ella U. Owens, with the editorial assistance of Helen Knight Steward. Price, $18.00. Pp. 489, with 260 plates. Published under the joint sponsorship of the American Academy of Ophthalmology and Otolaryngology and the Armed Forces Institute of Pathology. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1952.

This book, as its title proclaims, is both an atlas and a textbook of ophthalmic pathology and is intended as a reference book as well as a guide to those preparing for advanced examinations in ophthalmology. All these intentions have been amply achieved, for this is truly a magnificent and monumental book in which the sponsors and the authors can take great pride. It is likely to remain for many years the authoritative reference book on the subject.

The book begins with chapters on the physiology, normal anatomy, and histology of the eye, and on the effects of growth and aging. Then follow four chapters on inflammations of the eye and a chapter on injuries. Extrabulbar diseases; diseases of the conjunctiva, the cornea, and the lens; diseases of intraocular fluid circulation (glaucoma and hypotony); diseases of the blood vessels, retina, optic disc, and nerves; congenital and developmental anomalies; prenatal and neonatal diseases; heredofamilial and degenerative diseases, and tumors are all considered in detail.

Each chapter includes a systematic discussion of the lesions, a bibliography, and at the end a photographic atlas illustrating the pathological features.

The text, which is based largely on Friedenwald's "Pathology of the Eye," is up-to-date and treats the subject thoroughly yet concisely and with economy of language. The references are pertinent and will be helpful to anyone wishing to verify original sources or to extend his reading.

The photographic illustrations are superb. They have been well taken, intelligently labeled, and carefully reproduced. When I tell you that there are over 240 plates, each of which contains on the average 4 pictures, you can realize the magnitude of the task that has been done. Most of the pictures are photomicrographs and include many very low magnifications of sections of the whole eye. The variants of the different lesions are also adequately illustrated.

From the above comment it is easy to see that the reviewer recommends this book without reservation. In closing, the reviewer also would like to point out that this book is the fruit of intelligent cooperation between the American Academy of Ophthalmology and Otolaryngology and the Armed Forces Institute of Pathology and that this cooperation made possible the establishment of the Registry of Ophthalmic Pathology in which has been assembled the greatest collection of ophthalmic pathology in the world. The full extent of this collection, the foresight of its founders and custodians, and the use which they have made of it are now clear for all to see.

Der Tuberkuloseablauf im Körper. By Priv.-Doz. Deiner W. Müller. Price, 24 German marks. Pp. 170, with 55 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1952.

This volume deals with the various initial stages of tuberculosis and describes its cause, and propagation, and various outcomes, as occurring not only in man but also in cattle. The author's experience is based on a multitude of observations made both in the clinic and at the autopsy table. The author's writing is lucid. Amusing is his confession of having recognized millet seed cereal from his recollection of the size and shape of miliary tubercles.

The Pathology of Diabetes Mellitus. By Shields Warren, M.D., Sc.D., Departments of Pathology of the New England Deaconess Hospital and the Harvard Medical School, Boston, Director of the Division of Biology and Medicine, United States Atomic Energy Commission, and Philip M. LeCompte, M.D., Departments of Pathology of the Faulkner Hospital and the Harvard Medical School, Boston. Third edition. Price, $7.50. Pp. 336, with 112 illustrations and 3 colored plates. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1952.

This book, now in its third edition, is an authoritative summation of present knowledge and theory pertaining to diabetes mellitus. It contains background information of great value to clinicians and pathologists. In presenting observations drawn from over 800 autopsies on diabetic patients, the authors have achieved a remarkable perspective in problems related to the disease in patients of all age groups and in all its manifestations.

The descriptions of methods of preparation of slides and specimens remain one of the features of special interest to the pathologist. New material of general interest is contained in the section on medicolegal problems in diabetes and in a most interesting series of autopsies on infants of diabetic mothers. Recent developments in experimental diabetes and in the renal complications of the disease are also presented.

Atlas de anatomia patológica. By M. Ravelo Barré, professor of pathological anatomy of the University of Santo Domingo; director of the laboratory of pathological anatomy and histology of the faculty of medicine; corresponding member of the Cuban Society of Cancerology. Price, not given. Pp. 162, with numerous illustrations. Imprenta ABC, República Dominicana, 1949.

This atlas was written to serve as a guide for students at the University of Santo Domingo in their practical work in pathology. The text and illustrations center about the set of slides used by these students in their classes. The text is brief and is written in Spanish in clear

and simple style. As a rule, it adequately covers the subject matter. The illustrations are predominantly photomicrographs in black and white of the most instructive field in each slide. These photographs are of good quality and are well chosen.

Atlas of Tumor Pathology: Section III, Fascicle 9, Teratomas. By Rupert A. Willis, D.Sc., M.D., F.R.C.P., Professor of Pathology, University of Leeds Medical School, Leeds, England. Price, $0.50. Pp. 58, with 45 illustrations and 1 color plate. Published by the Armed Forces Institute of Pathology, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, 2101 Constitution Ave., N. W., Washington 25, D. C., 1951.

Most textbooks of pathology skirt around the subject of teratomas in cursory fashion. Willis' own volume on the pathology of tumors is a shining exception. The present fascicle presents a splendidly organized and illustrated analysis of this difficult subject in a readily accessible form. The text is clearly written, analytical, and critical. The illustrations stress gross photographs and low-power enlargements and are of high quality. The volume is highly recommended.

Practical Dermatology. By George M. Lewis, M.D., F.A.C.P., professor of clinical medicine (dermatology), Cornell University Medical College; attending dermatologist, The New York Hospital; secretary, the American Board of Dermatology and Syphilology. Price, $7.50. Pp. 328, with 99 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

Atlas of Tumor Pathology. Section VIII, Fascicles 31b and 32, Tumors of the Male Sex Organs. By Frank J. Dixon, M.D., Professor of Pathology, University of Pittsburgh School of Medicine, and Robert A. Moore, M.D., Professor of Pathology, Washington University School of Medicine. Price, $1.50. Pp. 179, with 135 illustrations. Published by the Armed Forces Institute of Pathology, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, 2101 Constitution Ave., N. W., Washington 25, D. C., 1952.

In this latest fascicle published by the Armed Forces Institute of Pathology, the tumors of the male sex organs are ably presented. Carcinoma of the prostate and the germinal tumors of the testis are especially well discussed. Nodular hyperplasia of the prostate as a "tumor-like lesion" is briefly presented, but a somewhat more detailed analysis might have been desirable. The presentation of material includes tumors of the testicular appendages, the urethra, Cowper's glands, the seminal vesicles, the penis, and the scrotum. The illustrations are uniformly of very excellent quality. Editorial error allowed "condyloma acuminatum" to be printed "condyloma acuminata" and permitted the solecism "condylomatas." Otherwise the text and legends are clear and informative, and the bibliographies are adequate. The volume is highly recommended.

Urine and the Urinary Sediment. By Richard W. Lippman, M.D., Research Associate, Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles; Fellow of the John Simon Guggenheim Memorial Foundation. Price, $7.50. Pp. 124, with 61 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1952.

Examination of the urinary sediment is a procedure which is often omitted by the busy practicing physician and is often not adequately taught to medical students. This small book (124 pages) fills a long-standing need for a clear exposition of the morphology and clinical importance of the urinary sediment. The morphology of the different clinically important varieties of casts is presented with the help of a large series of beautiful photomicrographs of urinary sediments from patients with many types of kidney disease. The author's views on the clinical significance of the various combinations of formed elements are similar to those of the late Thomas Addis, with whom he was associated for several years.

This would be an excellent book for medical students except for the relatively high price, which may prevent some of them from buying it. The format and quality of the publication are superior and well worth this price, however. This book should be read by every physician who treats patients with kidney disease.

Current Therapy 1953: Latest Approved Methods of Treatment for the Practicing Physician. Edited by Howard F. Conn, M.D., and 12 consulting editors. Price, $11.00. Pp. 835. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

Gynecologic and Obstetric Pathology, with Clinical and Endocrine Relation. By Emil Novak, M.D., D.Sc. (Hon., Trinity College, Dublin; Tulane), F.A.C.S., F.R.C.O.G. (Hon.), Assistant Professor Emeritus of Gynecology, The Johns Hopkins Medical School; Gynecologist, Bon Secours and St Agnes Hospitals, Baltimore; Fellow and Past President, American Gynecological Society. Third edition. Price, $10.00. Pp. 595, with 630 illustrations, 19 in color. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1952.

This popular textbook, to some extent, has been revised and expanded in its third edition. New illustrations, principally photomicrographs, have been added. Also, a closing chapter on common diseases of the breast has been included.

This book is of such a high degree of excellence that it may seem improper to criticize any part of it. Nevertheless, the reviewer believes that the drawings in the first chapter on "Diseases of the Vulva," which have been retained in the present edition, are superfluous. Likewise, the chapter on the breast might have been omitted since it seems oddly out of place and does not carry with it the depth of personal experience and authority which makes the rest of the text so valuable. In conjunction with the latter, there are at least two items to which attention should be drawn. The discussion of granuloma venereum properly should be brought up to date with respect to the etiologic agent, and a more illuminating photograph of the organisms should have been supplied. On the subject of so-called lymphangiomas or mesotheliomas of uterus and Fallopian tube, the alternative term adenomatoid tumor should be added. While this term brings us no closer to an understanding of the histogenesis of the lesion, it is used currently by general pathologists.

Despite these criticisms, this textbook can be highly recommended to clinicians and pathologists who deal with gynecological and obstetrical problems. For students, the volume remains a reliable and excellent text.

Atlas of Medical Mycology. By Emma Sadler Moss, M.D., and Albert Louis McQuown, M.D. Price, $8.00. Pp. 245, with 248 illustrations. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1953.

The contents are as follows: (1) Classification, (2) Actinomycosis, (3) Nocardiosis, (4) Mycetoma Pedis, (5) Trichomycosis Axillaris, (6) Erythrasma, (7) Cryptococcosis, (8) Moniliasis, (9) North American Blastomycosis, (10) South American Blastomycosis, (11) Histoplasmosis, (12) Sporotrichosis, (13) Coccidioidomycosis, (14) Occasionally Pathogenic Fungi, (15) Chromomycosis, (16) Dermatomycosis, (17) Rhinosporidiosis, (18) Tinea Versicolor, (19) Piedra, (20) Methods, (21) Immunology, (22) Contaminants, (23) Culture Media, (24) Glossary, (25) Formulary and References. Illustrations follow each chapter.

Each chapter is, so far as practicable, divided into etiology, definition of disease, distribution, clinical disease, x-ray diagnosis, laboratory diagnosis, mycology, histology, treatment, and prognosis.

The authors point out that mycological diagnosis is no more formidable than are definitive studies of bacteria. The problems have been simplified and clarified, and material has been condensed in an admirable fashion. The manner in which the material has been condensed is probably the most commendable feature of this book, for in all instances only pertinent matter is included.

The authors call attention to the fact that in fungus diseases definitive diagnosis (such as isolation, cultivation, and identification) is imperative and can be accomplished only in the laboratory. This is important, since the symptomatology may mimic other chronic diseases. To accomplish this in the most efficient manner they have suggested the use of a minimum amount of culture media which can be prepared easily in any laboratory.

The writers particularly recommend the use of Littman's ox gall agar as a routine selective medium for the primary isolation of pathogenic fungi. In general, medical mycologists agree that it is useful. However, the macroscopic and sometimes microscopic growth characteristics on this medium differ from those seen on Sabouraud's glucose agar. The authors have explained

that Littman's ox gall agar surpasses Sabouraud's agar as an identifying medium when one is familiar with the growth characteristics of the various pathogenic fungi. Growth patterns of those fungi considered are so fully described that one is soon able to recognize them. Thus, this book should be of value to clinicians, pathologists, and medical mycologists.

Functional Endocrinology from Birth Through Adolescence. By Nathan B. Talbot, M. D., Associate Professor of Pediatrics, Harvard University; Physician, Children's Medical Service, Massachusetts General Hospital; Edna H. Sobel, M.D., formerly, Research Fellow in Pediatrics, Harvard University; at present, Instructor in Pediatrics, University of Cincinnati College of Medicine; Research Associate, Children's Hospital, Research Foundation and the Fels Research Institute, Antioch College; Janet W. McArthur, M.D., Instructor in Gynecology, Harvard University; Assistant Physician, Massachusetts General Hospital, and John D. Crawford, M.D., Instructor in Pediatrics, Harvard University; Asssistant Physician, Children's Medical Service, Massachusetts General Hospital. Price, $10.00. Pp. 638, with numerous illustrations and tables. Published for the Commonwealth Fund by the Harvard University Press, Cambridge 38, Mass., 1952.

Ten chapters cover respectively the thyroid, the parathyroids, the adrenal cortices, the adrenal medullae, the ovaries, the testes, the anterior pituitary (this specifically in relation to growth), the posterior pituitary, and the pancreatic islets. Aspects of pituitary involvement with the various target organs are treated along with the targets themselves. The general plan has been to present basic considerations of the hormones involved, their determination and their physiological mechanisms, followed by a discussion of clinical disorders and their treatment. The former gives a good survey of the literature (where familiar to the reviewer); the text is necessarily somewhat streamlined and weighed, perhaps, especially concerning the parathyroids and adrenals, to give the views of the noted school of origin of this work. The clinical portions include well-chosen examples, good photographs, clear charts and tables, and the usual fuzzy photomicrographs proper to semismooth paper reproduction. In every way, this book should be valuable, for study and reference, to its audience of practitioners and students. The stated objective, to carry forward the theme of homeostatic change as enunciated by Cannon, is a worthy one, and if the thread may have been lost at times in the masses of detail, it is because these "stubborn facts" still await effective coordination.

Oral Fat Emulsions. By Frederick J. Stare, with 27 contributors. Price, $3.00. Pp. 139, with tables and charts. Ann. New York Acad. Sc. **56**:1-139 (Oct. 10) 1952.

Ionic Transfer in Nerve in Relation to Bio-Electrical Phenomena. A. M. Shanes, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service, Federal Security Agency, Bethesda, Md. Price, $1.00. Pp. 36, with charts and tables. Ann. New York Acad. Sc. **55**:1-36 (June 30) 1952.

Use of Antibiotics in Tropical Diseases. By H. W. Brown, and 50 contributors. Price, $4.00. Pp. 317, with charts and tables. Ann. New York Acad. Sc. **55**:967-1284 (Dec. 30) 1952.

Biology of the Testes. Edited by Warren O. Nelson, with 29 contributors. Price, $3.75. Pp. 198, with numerous illustrations. Ann. New York Acad. Sc. **55**:543-742 (Nov. 20) 1952.

American Pocket Medical Dictionary: A Dictionary of the Principal Terms Used in Medicine, Nursing, Pharmacy, Dentistry, Veterinary Science, and Allied Biological Subjects. Ninteenth edition. Price, $3.25 plain; $3.75 with thumb-index. Pp. 639. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

This new edition of the "American Pocket Medical Dictionary" has been revised to include new and up-to-date terms and to eliminate obsolete terms. The volume is compact and at the same time sufficient to the needs of persons working in fields related to medicine (dentistry, pharmacy, nursing, and allied biological subjects). New features of this edition are tables of bones and veins, the listing of plurals of medical terms which are frequently troublesome because

of their irregular formation, and the inclusion of combining forms or word-elements that provide clues to interpretation of new terms. The newly added tables, together with the rewritten tables of arteries, muscles, nerves, and chemical elements, provide useful information in compact form.

Pathology in Surgery. By Edwin F. Hirsch, Ph.D., M.D., Director of the Henry Favill Laboratory; Pathologist of St. Luke's Hospital, Chicago; Research Associate, Associate Professor (Emeritus) of the Department of Pathology of the University of Chicago. Price, $10.00. Pp. 474, with 388 illustrations. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1953.

The text, which comprises approximately one quarter of this volume, is tersely written, factual, and elementary. The tenor is wholly descriptive with little effort to relate the pathologic with the biologic. Relatively few references are appended to each of the eleven sections, and these are selected with a curious geographic bias.

The bulk of the volume is devoted to photographs, of which photomicrographs are in the majority. These are excellent and are crisply reproduced. Each photograph is labeled, but lesion characteristics are not delineated. The gross photographs exhibit technical superiority. They are uniformly prepared from fixed specimens, however, and in many instances are not revealing.

The book is, in essence, an album of well-prepared, representative photographic illustrations of pathologic lesions encountered in surgical pathology.

Diseases of Metabolism: Detailed Methods of Diagnosis and Treatment. Edited by Garfield G. Duncan, M.D., Director of Medical Division, Pennsylvania Hospital, and Clinical Professor of Medicine, Jefferson Medical College, Philadelphia. Third edition. Price, $15.00. Pp. 1,179, illustrated. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1952.

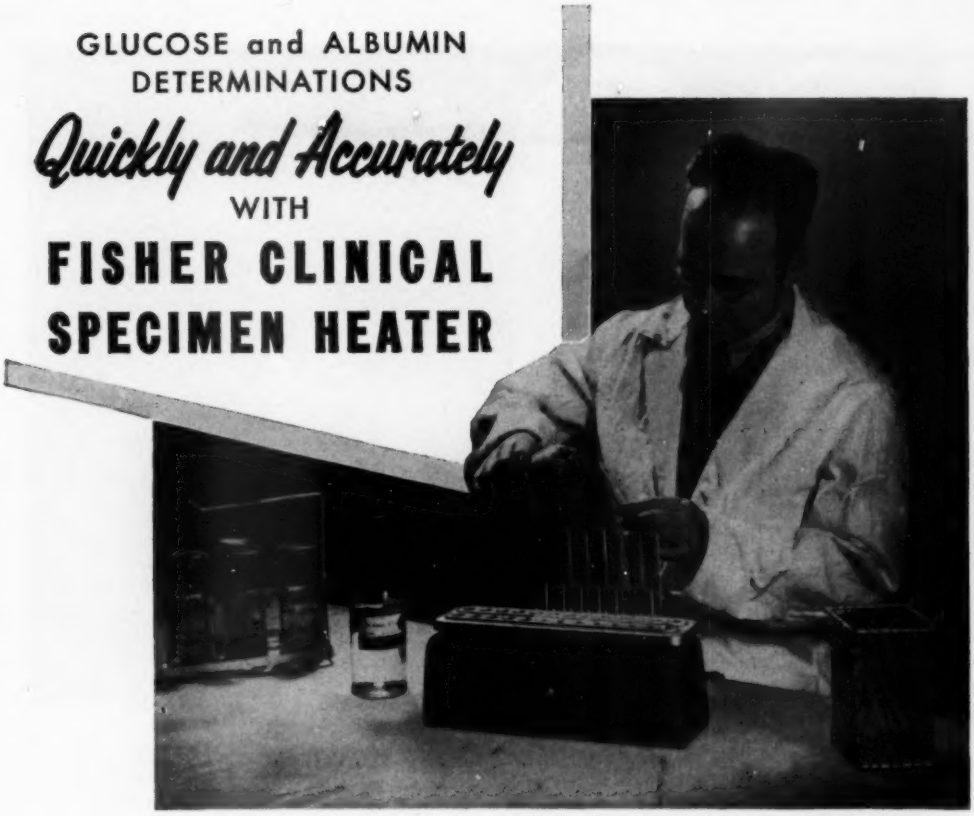
Popular textbooks reflect rather exactly the philosophy of their time, and the third edition of Duncan's "Diseases of Metabolism" mirrors our generation's interest in the metabolic aspects of health and disease. As a matter of fact, the title of this standard work is misleading, for the authors have not confined themselves solely to a consideration of diseases of metabolism. For the third edition, much of the book has been rewritten, and a chapter on "Porphyrin Metabolism" has been added. Unfortunately, the chapter on "Nutritional and Metabolic Aspects of Disorders of the Blood" has been dropped; there seems to be little or no discussion of hemoglobin breakdown in the present text, and the reviewer could not find a word on familial nonhemolytic jaundice, which is certainly a metabolic disease. This is surprising, since one of the contributors, Dr. C. J. Watson, has made notable contributions to our knowledge of the metabolism of bilirubin. However, these and other omissions do not detract from the usefulness of the book or from its authority. New contributors include a galaxy of stars, such as S. Soskin, R. Levine, C. J. Watson, and Ancel Keys. Their chapters are among the outstanding ones in the book. As usual, that master of medical prose, Dr. John P. Peters, has contributed a singular and lucid chapter on "Water Balance in Health and Disease," and the editor, Dr. G. G. Duncan, has brought his useful chapter on "Diabetes Mellitus" up to date. While comparisons are odious, the reviewer believes that the third edition of this text is better than the earlier texts and predicts that it will be more popular than ever with those who are interested in metabolic aspects of disease.

GLUCOSE and ALBUMIN DETERMINATIONS

Quickly and Accurately

WITH

FISHER CLINICAL SPECIMEN HEATER



The presence of glucose in a urine specimen is determined in the 13 openings in the back row of the instrument by Benedict's Qualitative method while the openings in the front row are provided for determining the presence of albumin by Purdy's test.

The Heater has a built-in thermostat that prevents it from becoming too hot. In back row the test tubes are heated at the bottom while in the front row they are heated about two inches above the bottom, or at a point corresponding approximately with the top of the liquid level as specified in the methods. Test tubes up to 16 mm. in diameter can be accommodated in both rows.

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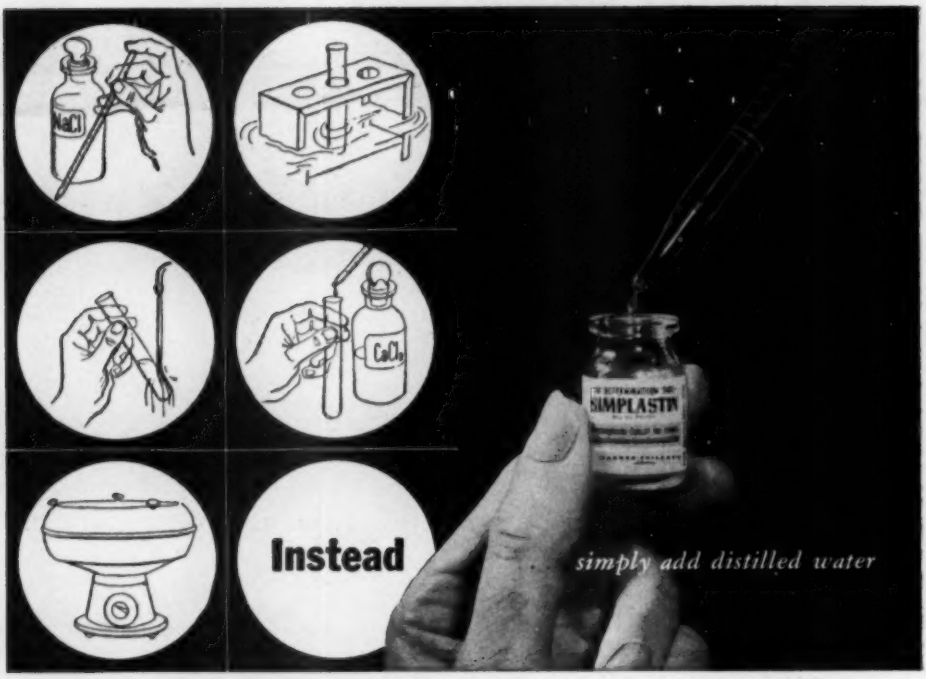

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Bibliography: 1. Shapiro, S., et al.: Am. Heart J. *40*:766 (Nov.) 1950. 2. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. *222*:207 (Aug.) 1951. 3. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:52 (Feb.) 1951.

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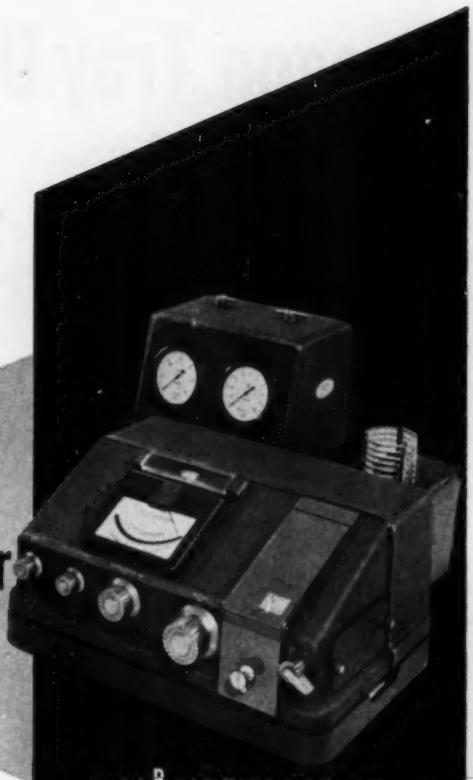

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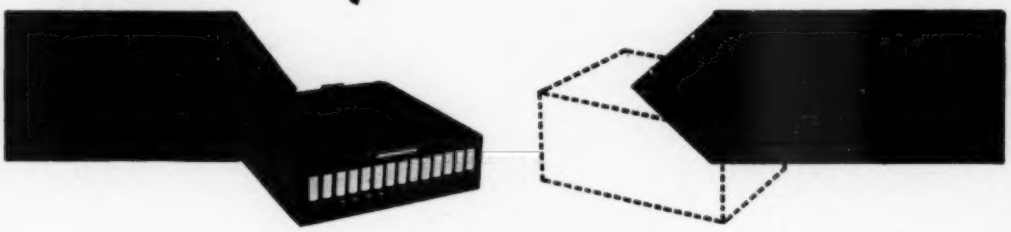

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